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ASSESSMENT OF CELL MEDIATED IMMUNE RESPONSE IN CORONARY HEART DISEASE USING SERUM ADENOSINE DEAMINASE ACTIVITY.

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ABSTRACT

Coronary heart disease is the major killer disease worldwide. Atherosclerosis is considered as the main cause of the disease. Atherosclerosis is a multifactorial, multistep disease that engrosses chronic inflammation at every step. Earlier studies suggested that, immunological responses contributing to inflammation play a vital role in plaque rupture and thrombosis causing stroke. Present study was one such attempt to estimate the level of adenosine deaminase activity as an immunoenzyme marker with additional enzymatic biomarkers like lactate dehydrogenase and aspartate aminotransferase in coronary heart disease like stable angina, unstable angina and myocardial infarction. In the present case-control study, 55 patients with stable angina, 100 patients with unstable angina and 110 patients with myocardial infarction (age range 26 to 75) and 120 age and sex matched healthy controls were recruited. Serum adenosine deaminase activity was evaluated by Gusti and Galanti method using digital spectrophotometer. Serum lactate dehydrogenase was measured by Optimized DGKC, Kinetic Assay and aspartate aminotransferase was estimated by modified UV kinetic assay using semiautoanalyser. Values were expressed as mean ± standard deviation. Comparisons of study groups and study groups to control group were done by applying Z test. ANOVA test and Tukey-Kramer test of multiple comparisons were applied to compare all groups together in respect to all parameters under study. Serum adenosine deaminase level was increased significantly ($p<0.01$) and serum levels of lactate dehydrogenase along with aspartate transaminases were enhanced significantly ($p<0.01$) in all types of coronary heart disease as compared with healthy controls. By applying one way ANOVA test, (value of 'F' = 499.52 $p<0.01$) and Tukey-Kramer multiple comparison test ($p<0.01$), the means of the mean values of enzymatic status in all types of coronary heart disease and controls were significantly differs by chance. In present study, elevated adenosine deaminase activity had seen in all types of coronary heart disease than healthy controls. Adenosine deaminase levels are elevated whenever cell mediated immunity is stimulated. Thus adenosine deaminase may be an important immunoenzyme marker in assessing the cell mediated immuno response in coronary heart disease for better management and for developing new treatment strategies. Mean serum lactate dehydrogenase and aspartate aminotransferase levels were superior in stable angina, unstable angina and myocardial infarction than healthy controls. Lactate dehydrogenase and aspartate aminotransferase can be used as biomarkers along with recent cardiac biomarkers, in diagnosis and prognosis in coronary heart disease.

KEY WORDS: Coronary heart disease, Stable angina, Myocardial infarction, Unstable angina, Adenosine deaminase, Lactate dehydrogenase and Aspartate aminotransferases.

INTRODUCTION

Coronary heart disease (CHD) is defined as acute or chronic cardiac disability arising from imbalance between the myocardial supply and demand for oxygenated blood.[1]

It is multifactorial in etiology and has spectrum of presentations ranging from Stable Angina (SA), acute coronary syndrome to completely asymptomatic disease.[2]

Cardiovascular disorders (CVD) are the leading cause of morbidity and mortality both in developed and developing countries. It has been predicted that, CVD will be the most important cause of mortality in India by the year 2015. CHD is the single most important contributor to this increasing burden of CVD.[3]
It is the largest killer disease in developed countries and is rapidly assuming a similar role in developing countries. The World Health Organization (WHO) has

drawn attention to the fact that CHD is our modern epidemic not an unavoidable attribute of aging.[4] The burden of CHD is rising in India. According to WHO, 7.2 million deaths i.e. (12.8% of total deaths) of CHD occured in 2008.[5]

In the etiology, CHD is caused by disease affecting the coronary arteries. More than 90% cases caused by coronary atherosclerosis and 10% by other causes like vasospasm, stenosis of coronary ostia, arteritis, embolism, thrombic diseases like shock, polycythaemia and trauma.[6]

'Adenosine', a degradation product of Adenosine Triposphate (ATP), acts as an endogenous cardioprotective agent in conditions such as myocardial ischemia. It also acts as coronary artery vasodilator to increase the blood supply to ischemic myocardium. Adenosine levels are regulated by the activity of the enzyme Adenosine Deaminase (ADA) E.C. 3.5.4.4 which is a cytosolic enzyme of the purine catabolic pathway.[2]

The major sources of serum ADA may be lymphocytes or the monocytes microphage cell system. It is required for lymphocyte proliferation and differentiation so that it is considered as important 'immumo'enzyme marker in assessing the cell mediated immune response. Estimation of ADA activity in Myocardial Infarction (MI) is important marker and plays a vital role in assessing the activation of T- lymphocyte in the pathogenesis of MI.[7, 8] Enhanced activity of ADA in serum can be used as molecular marker of the evaluation in acute MI patients who had not receiving thrombolytic therapy and presenting no changes in the ST- segment.[9]

Thus, in view of above information and several risk of complication, it is worthwhile to study the enzymatic status in CHD. Very few studies have been reported from serum ADA level in CHD. Acquaintance of enzymatic status in CHD may help in planning proper strategies in clinical management of the disease. Hence aim of present study was to find ADA activity in patients with different types of CHD like stable angina, unstable angina, and myocardial infarction and to compare it with healthy controls. At the same time, study was designed to assess Aspartate Amino Transferase (AST), and Lactate Dehydrogenase (LDH) levels in CHD and to determine its association with the extend of CHD.

MATERIAL AND METHODS

The present case-control study was conducted at Department of Biochemistry, PDVVPF's Medical College, Ahmednagar and Swasthya Hospital and Research Centre Ahmednagar (Maharashtra) in collaboration with Department of Biochemistry, B. J. Medical College and Sassoon General Hospital (S.G.H) Pune. The study was approved by Ethics Committee of B.J.M.C. and S.G.H. Pune with all participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Study type: Diagnostic case control study

Study design: Total 385 samples were enrolled in the present study.

Control group: 120 healthy age and sex matched individuals without any evidence of CHD as per clinical examinations were taken as control subjects.

Patients Group: The study included total 265 patients between age group 26 to 75 years of CHD. Of these, patients of Myocardial Infarction (n= 110) and Unstable Angina (n=100) had taken from Intensive Cardiac Care Unit (ICCU) having chest pain.

Patients of stable angina (n=55) had taken from outpatients attending the cardiology department of same hospitals.

The patients were diagnosed by physicians blinded to the results of markers; data included history, physical examination, serial 12-lead electrocardiogram and cardiac markers measurement.

Inclusion Criteria: The diagnosis of all patients of CHD had made by physicians and patients had typical chest pain, specific abnormalities for CHD on electrocardiogram, elevated cardiac markers were included.

Exclusion criteria: All patients having history of heart disease like congenital heart disease, diseases of heart valves & myocardium. Confounding factors which could interfere in the biochemical analyses of study groups and alter the results were diabetes mellitus, renal insufficiency, hypertension, hepatic disease, inflammatory disease, history of recent infection, febrile disorders.

Collection of specimen: Blood sample collection was different for different groups. For control and SA, 3ml blood was collected between 9.00 to 11.00 am after fasting from 10.00 pm of previous day by using 20G disposable needle from cubital vein with asceptic precaution. For MI and UA, 3ml blood was collected within 6 hours to 12 hours after admission in the ICCU. Plain Vaccutainer (Yucca Diagnostic) was used for estimation of AST, LDH, and ADA. After an hour, the samples were centrifuged at 3000 rpm for 10 minutes to separate serum. The separated serum was collected in polythene tube with cork and stored at room temprature (precaution were taken to avoid the hemolysis) and used for analysis with 8 hours.

Method

1) Determination of serum ADA activity: The ADA levels measured spectrophotometrically by (Gusti and

Galanti) method based on the berthlot reaction that is the formation of colored indophenol complexes from ammonia liberated from adenosine. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the serum sample.[10]

2) Determination of serum LDH activity: Serum LDH activity was estimated by optimized DGKC,kinetic Assay method by commercially avilable kit(Span diagnostic) using semiautoanalyser.[11]

3) Determination of serum AST activity: Serum AST Activity in serum was calculated by modified UV Kinetic Assay by commercially available kit using semiautoanalyser on 340nm.[12]

Statistical analysis

Statistical software SYSTAT version-12 (by Cranes software, Bangalore) was used to analyze the data. The results were expressed in Mean ± Standard Deviation (Mean ± SD).

Data was analysed by descriptive statistics as mean, SD, percentage etc. Comparisons of study groups and study groups to control group were done by applying Z test of difference between two sample means at 5% (p, 0.05) and 1% (p, 0.01) level of significance.

ANOVA test and Tukey-Kramer test of multiple comparisons was applied to compare all groups together in respect of all parameters under study.

RESULT

Baseline characteristic of CHD patients and controls are shown in Table No.1. By applying 'Z' test of difference between two means, there was a significant difference between mean pulse rate, diastolic blood pressure, systolic blood pressure, and body mass index when healthy control group compared with all CHD groups as stable angina, unstable angina and myocardial infarction individually ($p<0.01$) and also there was a significant difference ($p<0.01$) between mean pulse rate, diastolic blood pressure, systolic blood pressure, and body mass index when all CHD groups compared with each other.

By applying one way ANOVA test, (value of 'F' = 577.08, $p<0.01$) and Tukey-Kramer multiple comparison test ($p<0.01$), the means of baseline characteristics such as pulse rate, diastolic blood pressure, systolic blood pressure, and body mass index in CHD and controls were significantly differs by chance. (Table No.2)

As shown in Table No.3, Serum adenosine deaminase along with lactate dehydrogenase and aspartate transaminases were enhanced significantly ($p<0.01$) in all types of CHD as compared with healthy controls. By applying 'Z' test of difference between two means there was significant difference ($p<0.01$) between mean values of enzymatic status as ADA, AST, and LDH when healthy control group compared with all CHD groups as stable angina, unstable angina and myocardial infarction individually and there were significant difference ($p<0.01$) between mean values of enzymatic status as like ADA, AST, and LDH when all CHD groups compared with each other.

By applying one way ANOVA test, (value of 'F' = 499.52 $p<0.01$) and Tukey-Kramer multiple comparison test ($p<0.01$), the means the mean values of enzymatic status as ADA, AST, and LDH in CHD and controls are significantly differs by chance ($p<0.01$). (Table No.4).

Table No.1: Baseline characteristics in CHD and controls

Variable	Controls (n=120)	CHD		
		Stable Angina(n=55)	Unstable Angina (n=100)	Myocardial Infarction(n=110)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Pulse rate	71.65 ±2.09	71.53 ±2.09	85.80 ±18.39	87.73 ±18.31
Diastolic blood pressure	74.83 ±5.79	75.66 ±9.79	84.40 ±18.38	84.95 ±21.71
Systolic blood pressure	112.25 ±8.54	117.74 ±11.54	131.63 ±30.35	132.48 ±27.25
Body Mass Index (BMI)	22.52 ±2.94	25.22 ±2.56	24.65 ±2.72	24.07 ±2.87

Table No 2: ANOVA TABLE of baseline characteristics in CHD and controls

Source of variation	d. f.	Sum of squares	Mean square
Treatment (between columns)	15	2015980	134399
Residuals (within columns)	1520	354000	232.89
Total	1535	2369980	

Value of 'F' = 577.08, $p<0.01$, highly significant

Table No.3: Enzymatic Status in Controls and CHD

Variable	Controls (n=120)	CHD		
		Stable angina (n=55)	Unstable Angina (n=100)	Myocardial Infarction (n=110)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ADA (U/L)	15.76±6.88	41.97±15.03*	51.82±18.81*	58.80±16.75*
AST (IU/L)	13.16±4.91	17.81±8.96*	38.54±29.56*	53.41±21.89*
LDH (IU/L)	280.55±78.56	304.99±83.19*	479.70 ±171.87*	694.13±279.27*

Values were expressed in mean with Standard Deviation (Mean±SD),
***** $p < 0.001$-- considered as highly significant,

Table No.4 ANOVA TABLE of enzymatic status in Controls and CHD

Source of variation	d. f.	Sum of squares	Mean square
Treatment (between columns)	13	57200000	4402214
Residuals (within columns)	1406	123900000	8812.8
Total	1419	181100000	

Value of 'F' = 499.52, $p<0.01$, highly significant

DISCUSSION

The primary cause of CHD is mainly atherosclerosis. CHD occurs due to atheromatous narrowing and subsequent occlusion of the coronary arteries.[6] Characterization of human atheroma has determined that, T- cells localize to lesions early during pathogenesis. Activated macrophages, T-lymphocytes and mast cells were found in the atherosclerotic arterial wall and thus the thrombotic component of the disease is attributed by immunological processes of the cellular and humoral type.[13]

The major mechanisms by which T-cell contribute to the pathogenesis of inflammatory disease are via the release of specific pattern of cytokines. Evidence indicates for the low density lipoprotein induced pathway of type I cytokine activation in atherosclerosis is regulated by the local production of interleukin (IL)-10 and IL-12. Thus the degree of T-cell and macrophage infiltration in atherosclerotic plaques has been shown to positively correlate with the occurrence of plaque rupture.[14]

It is well established that, the ADA levels are elevated whenever cell mediated immunity is stimulated and thus reflects the activity of stimulated T-lymphocytes. Formation of cytokines also regulates membrane adenosine deaminase on human lymphocytes. Interferon gamma activates the monocytes macrophage cell system which may also contribute to the regulation of plasma ADA activity.[15] Hence ADA level in CHD has been estimated to assess the cell mediated immune response.

The results of the present study (Table No 3) showed significantly elevated ($P<0.01$)) mean levels of ADA in stable angina, ustable angina and myocardial infarction patients when compared to controls. There was a significant difference ($p<0.01$) between mean values of ADA when all CHD groups compared with each other. By applying the ANOVA test and Tukey-Kramer multiple comparison test, the means of ADA in all types of CHD and controls were significantly differs by chance ($p<0.01$).

Our results were strongly supported to previous studies[7, 8, 9] where ADA level was significantly elevated in CHD. Yulan Torrelles et al have demonstrated that, elevation of ADA in serum of ischemic patients suggests an adaptive metabolic phenomenon where high levels of adenosine are produced during tissue hypoxia. They also confirmed that ADA level can be used as a molecular marker of the evolution in acute MI patients receiving no thrombolytic therapy and presenting no changes in the ST-segment.[9]

Maria Kopff et al analysed the adenosine catabolism by ADA in plasma, erythrocytes, granulocytes and lymphocytes of patients with stable angina pectoris. According to them, the lowered ADA activity in granulocytes and lymphocytes of patients with stable angina pectoris representing less intensive catabolism of adenosine which cannot be interpreted univocally. It might suggest that, adenosine preserved in these blood cell is directed to ATP synthesis required for activation of polymorphonuclear leukocytes which in turn are trapped in the coronary circulation and are infiltrating the ischemic region. This may induce inflammatory reaction and clear up the irreversibly damaged and necrotic tissue.[16]

Vishwas Chawan et al have studied leukocyte hydrolytic enzymes in patients with acute stage of CHD. According to their study, ADA was more active in CHD patients and may contribute to inflammation related with

CHD.[17] Jyothy A et al estimated that, measurement of ADA activity in MI is important and may play a vital role in accessing the activation of T- lymphocytes in the pathogenesis of MI.[7] A highly significant complex relationship has emerged among ADA, birth weight and gender concerning their role for susceptibility to CAD in adult life.[18]

Dipyridamole an inhibitor of adenosine transport and ADA has been investigated as a possible candidate for the treatment of angina pectoris which indicates that ADA may have a crucial role in myocardial ischemic disease.[19] Aiki Kaul et.al have suggested that ADA has an important implication in ischemic myocardial syndrome.[2] Inhibition of ADA might be a novel and viable therapeutic approach to manage the systemic inflammatory response in CHD.[19]

LDH is an enzyme of anerobic glycolysis that catalyzes the reversible interconversion of lactate and pyruvate. It is widely distributed in all human tissues therefore its plasma level is elevated in a wide variety of diseases including MI. AST is formally known as Glutamate Oxaloacetate Transaminase or GOT catalyses the reversible transfer of the amino group from glutamate to oxaloacetate and form α-ketoglutarate and aspartate.

AST has a wide distribution being present in heart, liver, kidney, skeletal muscles and erythrocyte. AST is elevated in diseases involving these organs. In MI, gross elevations of AST activities are seen.[20,21] Hence additional aspire of this study was to revise serum levels of myocardial enzymes in various types of CHD like SA, UA, MI and normal healthy controls.

Present study showed that highly significant ($p<0.01$) mean levels of LDH and AST in SA, UA and MI as compared with healthy controls. In the same way, there was a significant difference ($p<0.01$) between mean LDH and AST when all CHD groups compared with each other. By applying the ANOVA test and Tukey-Kramer multiple comparison test, the means of LDH and AST in all types of CHD and controls were significantly differs by chance ($p<0.01$). Visileios Peppes et al have suggested that increased serum levels of myocardial enzymes with coronary artery disease in Greek patients. Our results were precisely matched to this outcome.[22] In cohort study**,** Kopel E et.al have verified that increased normal range of total serum LDH is associated with reduced short term risk of CAD outcome in low risk, physically active population.[23] M.R.Abdullah has demonstrated in his study that, prolonged ischemia originates accumulation of non esterified fatty acids intra and extra cellularly, which might change the permeability of plasma membrane of heart which may lead to the leakage of cellular substance and enzyme outside the cells.[24]

Gama R and Swain DG measured serum AST on three consecutive days after admission in acute myocardial patients and confirmed that AST had a diagnostic sensitivity of 100% and specificity of 86% although electrocardiograms of first two days of admission had a low diagnostic sensitivity (33.3%).[25] In a further follow up study, found that increased serum AST levels at peak value on 7th day after acute MI**.**[26]

CONCLUSION

The role of ADA may be as an important immunoenzyme marker in assessing the cell mediated immuno response in CHD for better management and for developing new treatment strategies. Study also specifies ADA along with LDH and AST can be important in the diagnosis of CHD. This study data may be proved that, these changes might be helpful to obtain a comprehensive view of the infarct size and severity of vascular stenotic lesions.

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