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Estimation of Serum Homocysteine , Adenosine Deaminase And Hs-CRP In Myocardial Infarction Patients “

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Abstract: *MI is almost always due to formation of occlusive thrombosis at the site of rupture or erosion of an atherosclerosis plaque in coronary artery . India is experiencing an alarming increase in heart diseases . CVD accounted for 32% of all deaths in 2000 and WHO estimates that 60% of worlds cardiac patients will be Indians by 2020 with CHD being the dominant form of CVD in India (1) . There is a considerable increase in prevalence of CHD in urban areas in India during the last decade . The pooled estimates that studies carried out in 1990's upto 2002 shows the prevalent rate of CHD in urban areas as 6.4% and 2.5% in rural areas . In urban areas the pooled estimate was 6.1% for males and 6.7% for females (2) . In rural areas the estimate was 2.1% for males and 2.7% for females. The principal risk factors which are not modifiable are age , sex ,family history , genetic factors where as the modifiable factors are behavioral factors (tobacco , diet , physical inactivity) , metabolic factors (lipid levels , obesity , hypertension , diabetes mellituss) .*

Despite the lake of agreement, however, continuous focus on newer factors is warranted as they may further improve our ability to predict future risk and determine treatment when they are included along with the classic risk factors. The study of these risk factors is important since the ability to accurately predict the CAD risk of a specific individual based on his or her conventional risk factor profile is limited. These newer risk factors are called 'Novel risk factors' which includes LP(a), homocysteine, fibrinogen, and hs-CRP .

Keywords: *MI – myocardial infarction , CVD - cardiovascular diseases , CHD - coronary heart disease , WHO – world health organization .*

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I. Introduction

Myocardial infarction (MI) represents end of acute coronary syndrome continuum, in which ischemic injury is irreversible, leading to cell death and necrosis. During the transition phase of 20th century, cardiovascular diseases became the most common. cause of death world wide. Approximately one million patients in a year suffer from MI in the United States. Driven by industrialization, urbanization and associated life style changes, this on going transition is occurring around the world among all races, ethnic groups and cultures at a faster rate. The term Acute Myocardial Infarction (AMI) is defined as an imbalance between myocardial oxygen supply and demand resulting in injury to and the eventual death of myocytes. Almost all MI s result from coronary atherosclerosis generally with superimposed coronary thrombosis. The clinical diagnosis of MI requires an integrated assessment of the history in combination with indirect evidence of myocardial necrosis using biochemical, electrocardiogram (ECG) and imaging modalities. Reports from World Health Organization(WHO) and American Heart Association (AHA) require the presence of at least 2 of the following for diagnosis of MI:characteristic symptoms, ECG changes and a typical rise and fall in biochemical markers. The Framingham Heart study is a land mark study in cardiovascular risk factors epidemiology. The version of Framingham risk point scores by NCEP is based on the traditional risk factors age, sex, dyslipidemia, blood pressure and smoking. The more recent prospective cardiovascular munstar(PROCAM) simple scoring scheme, 8 risk variables are identified: 1)Age 2)Family history of premature myocardial infarction, 3) Diabetes Mellitus 4) Systolic blood pressure 5) smoking 6) LDL-Cholesterol, 7) HDL-Cholesterol 8) Triglycerides. Despite the lake of agreement, however, continuous focus on newer factors is warranted as they may further improve our ability to predict future risk and determine treatment when they are included along with the classic risk factors. The study of these risk factors is important since the ability to accurately predict the CAD risk of a specific individual based on his or her conventional risk factor profile is limited. These newer risk factors are called 'Novel risk factors' which includes LP(a), homocysteine, fibrinogen, and hs-CRP Homocysteine is a sulfur containing amino acid, derived from the metabolism of dietary methionine. Elevated plasma homocysteine may be an important cause for atherosclerosis formation. The adverse effects of homocysteine, involve oxidative damage to vascular endothelial cells, increased proliferation of smooth muscle cells, and oxidative modification of low density lipoprotein, all leading to atherosclerosis. Hs-CRP is an acute — phase plasma protein belonging to the family of proteins known as pentraxins that is synthesized and released primarily by hepatocytes. The hs-

CRP has been demonstrated to actively contribute to all stages of atherogenesis, participating in endothelial dysfunction, atherosclerotic plaque formation, plaque maturation, plaque destabilization and eventual rupture. Patients with suspected acute myocardial infarction require immediate medical care, so it requires very early clinical detection to permit early therapy such as thrombolysis, to establish reperfusion of the area at risk, salvage of much as ischemia as possible, and consequently minimize infarct size because myocardial necrosis begins at approximately 30 min after coronary occlusion and extensive damage occurs with in 2 to 4 hours of ischemia.

II. Aim Of The Study

The aim of the study is to identify the elevated levels of

1. Homocystiene
2. hs-CRP
3. Adenosine deaminase as causative factors in endothelial and vascular damage particularly coronary arteries.
4.

III. Material And Methods

The present study is carried out in the department of biochemistry and department of Medicine, S.V.S. Medical College and Hospital, Mahabubnagar. Study was on Ischemic Heart Disease patients, (< + 55 yrs) with myocardial infarction, admitted in Cardiology department. A total number of 50 subjects have been included in the present study. These subjects have been grouped in to

Healthy controls - 25

MI subjects - 25

COLLECTION OF BLOOD SAMPLES: In each subject about 5 ml of blood is collected under aseptic conditions in emergency department with in 4 hrs of admission. Dispended in to clean dry test tube and allowed to clot. Serum is obtained with out any hemolysis. In these patients the following estimations were done in serum sample.

a) Homocysteine

b) hs-CRP

c) adenosine deaminase (ADA)

A)ESTIMATION OF HOMOCYSTEINE

1) METHOD Homocysteine competitive immuno assay using direct chemiluminescence technology.

2) EQUIPMENT : Auto analyzer.

3)NORMAL REFERENCE VALUE : Normal fasting plasma HCy levels in adults 5-15pm with a mean level of about 10 µm.

B)ESTIMATION OF ADENOSINE DEAMINASE

1)METHOD: Spectrophotometric method of Giusti and Galanti

2)REFERENCE VALUES: In serum / plasma - < 30 U/L

C)HS-CRP ASSAY

1)Method : Chemiluminescence immunoassay (CLIA)

2)Instrument: Lumax CLIA Strip Reader

3)Specimen required: Serum (fasting and postprandial samples)

4)Reference range : Serum : 1-3ng/ml

IV. Results

TABLE – 1

SERUM HOMOCYSTEINE, ADA AND hs-CRP LEVELS IN CONTROLS

S. No.	AGE/SEX	Troponin	Homocysteine	ADA	Hs-CRP
			µ Mols	Units/Lt.	ng/ml
1	55/M	Negative	7.2	21.24	0.93
2	58/M	Negative	8.1	12.8	1.1
3	62/F	Negative	7.8	10.66	1
4	60/M	Negative	6.3	18.2	0.99
5	63/M	Negative	8.2	18.85	1.67
6	65/M	Negative	7.8	14.65	2.36
7	62/M	Negative	7.6	13.78	1.84
8	60/M	Negative	8	17.62	0.98
9	58/F	Negative	7.9	21.4	1.68
10	56/F	Negative	11.67	24.47	2.27
11	56/M	Negative	7.5	18.6	1.86

12	61/M	Negative	6.8	16.8	1.28
13	64/M	Negative	10	16.45	1.88
14	65/F	Negative	6.6	17.42	2.2
15	65/M	Negative	6.8	21.5	0.85
16	68/M	Negative	5.5	18.95	0.99
17	59/M	Negative	5.6	13.26	1.22
18	67/F	Negative	6.4	14.28	2.76
19	69/F	Negative	7.2	15.43	1.1
20	70/M	Negative	8	13.23	0.97
21	56/M	Negative	7.4	10.52	1
22	69/F	Negative	6.8	11.8	1.23
23	63/M	Negative	7	12.92	2.63
24	72/F	Negative	8.2	16.73	1.86
25	70/M	Negative	6.4	18.12	2.86
Total			186.75	409.68	39.51
Mean			7.47	16.39	1.58
SD			1.29	3.63	0.63

SD-Standard Deviation

TABLE – 2

SERUM HOMOCYSTEINE, ADA AND hs-CRP
LEVELS IN Cases

S. No.	AGE/SEX	Troponin	Homocysteine	ADA	Hs-CRP
			µ Mols	Units/Lt.	ng/ml
1	59/M	Negative	7.2	21.24	0.93
2	67/F	Negative	8.1	12.8	1.1
3	69/F	Negative	7.8	10.66	1
4	70/M	Negative	6.3	18.2	0.99
5	56/M	Negative	8.2	18.85	1.67
6	69/F	Negative	7.8	14.65	2.36
7	63/M	Negative	7.6	13.78	1.84
8	72/F	Negative	8	17.62	0.98
9	70/M	Negative	7.9	21.4	1.68
10	56/F	Negative	11.67	24.47	2.27
11	68/M	Negative	7.5	18.6	1.86
12	59/M	Negative	6.8	16.8	1.28
13	67/F	Negative	10	16.45	1.88
14	69/F	Negative	6.6	17.42	2.2
15	70/M	Negative	6.8	21.5	0.85
16	56/M	Negative	5.5	18.95	0.99
17	59/M	Negative	5.6	13.26	1.22
18	62/F	Negative	6.4	14.28	2.76
19	55/F	Negative	7.2	15.43	1.1
20	57/M	Negative	8	13.23	0.97
21	60/M	Negative	7.4	10.52	1
22	69/M	Negative	6.8	11.8	1.23
23	67/F	Negative	7	12.92	2.63
24	65/M	Negative	8.2	16.73	1.86
25	62/M	Negative	6.4	18.12	2.86
Total			1720.23	1258.09	955.36
Mean			68.80	48.28	38.21
SD			15.70	12.55	9.48

SD-Standard Deviation

TABLE -3

SUMMARY OF RESULTS

S. No.	Investigations	Values	Controls	MI Cases
1	Homocysteine	Mean	7.47	68.80
		±SD	1.29	15.70
		t-Value	19.46	
		p-Value	0.0001	
2	ADA	Mean	16.39	48.28
		±SD	3.63	12.55
		t-Value	12.19	
		p-Value	0.0001	
3	Hs-CRP	Mean	1.58	38.21
		±SD	0.63	9.48
		t-Value	19.25	

		p-Value	0.0001	

SD Standard Deviation

Table 1 and 2 shows the clinical data and biochemical parameters of control and cases respectively . The mean and standard deviation comparison of all the three parameters are shown in above tables. The p- value of all the three parameters is less than 0.0001 which is considered as extremely significant. Table 3shows the summary of results comprising the mean , SD, t- value and p- value of serum homocysteine , ADA and hs-CRP of both cases and controls .

V. Discussion

Myocardial infarction is the leading health problem all over the world. So it has become important to achieve early diagnosis for MI. In the present study novel risk factors like homocysteine and hs-CRP and also adenosine deaminase are also included. In 25 cases of myocardial infarction which are proven by clinical history, ECG and positive Troponine test Homocysteine, hs-CRP and adenosine deaminase are carried out as per the standard procedures indicated.

All cases have extremely significantly elevated levels of Homocysteine, hs-CRP and Adenosine deaminase.

Homocysteine is a sulphur containing aminoacid, which is derived from the dietary methionine, has been associated with cardiovascular events. Homocysteine has been recently identified as a noval risk factor for coronary artery disease. Mc Cully made the first correlation between high homocysteine and vascular disease. Mc Cully KS. (1969). Vascular pathology of homocysteine: implications for the pathogenesis of arteriosclerosis. Later in 1976, Wilken et al published the first report that patients with coronary heart disease have abnormal homocysteine after 1990 many publications implicated

elevated homocysteine as an independent risk factor. In 1992 the first ever-positive prospective study was made on high plasma homocysteine as a risk factor in myocardial infarction among the physicians in United states. Another study in UK established that plasma homocysteine concentrations were higher among Asian Indians and higher fasting homocyeteine levels increased the mortality of the patients.

Hs-CRP is an acute phase plasma protein belong to the family of protein known as pentraxins that is synthesized and released primarily by hepatocytes.

The role of Hs-CRP a causative factor, in coronary artery disease is through promoting atherosclerosis. It arises from the combination of endothelial dysfunction and inflammation. The maintenances of vascular homeostasis depends on a balance between endothelium derived relaxing and contracting factors. With disruption of this balance the vasculature becomes susceptible to atheroma formation.

Adenosine deaminase is an enzyme of purine nucleotide catabolism is closely associated with T-lymphocyte function which is responsible for cell mediated immunity. Adenosine deaminase levels in serum and other biological fluids is most often used as marker of cell mediated immunity of T- lymphocyte activation. ADA levels in plasma and other biological fluids, are increased in tuburculosis, rheumataoid arthritis and leprosy and these diseases are characterized by chronic inflammation and involvement of cell mediated immune response.

In case of myocardial infarction, atherosclerotic process of arterial wall is the most important underlying predisposing factor for coronary thrombosis. Many studies on etiopathogenesis of atherosclerosis have shown in addition to elevated lipid levels, inflammation of arterial wall, subsequent immunological response play an important role.

From the present study on serum homocysteine, hs-CRP and ADA levels in myocardial infarction, it is concluded that in addition to routine cardiac markers in MI serum homocysteine, hs-CRP and ADA levels are also useful, as significant elevation of these markers are found in all cases.

VI. Conclusion

Coronary artery disease (CAD) is the greatest killer of mankind. The identification of major risk factors and effective control of these through population based strategies of prevention can help to decline the mortality due to CAD. As myocardial infarction will be the major component of CAD, it requires early diagnosis, to reduce mortality. Increased levels of homocysteine, hs-CRP and ADA were associated with myocardial infarction. In this context, estimation of novel risk factors like homocysteine, hs-CRP and immuno inflammatory marker ADA are useful in early diagnosis of myocardial infarction. Estimation of hs-CRP is useful in predicting the future CAD and initiating prophylactic measures and treatment.

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