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A study of homocysteine, creatine kinase and lipid levels in young patients with ischaemic heart disease

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Abstract:

Ischemic Heart Disease forms one of the commonest causes of morbidity and premature death in the community, both in India as well as abroad especially in the young aged individuals. The incidence of this disease is about 9.7% of the total population globally. Advancing Age, Family history of premature myocardial infarction, Diabetes Mellitus, Systolic Blood Pressure, Smoking, Increased LDL-Cholesterol levels, Decreased HDL-Cholesterol and hypertriglyceridemia are the well known risk factors which will predispose for the coronary artery disease. Certain novel risk factors such as Lipoprotein-A, Homocysteine, fibrinogen and C - reactive protein are also found associated with the incidence of Ischemic Heart disease. Estimation of these risk factors will pave for early detection and prompt management of Ischemic Heart Disease and its complications. The current study aimed at determining the relationship between total plasma homocysteine as an independent causative agent of ischemic heart disease in young patients and also of elevation of serum cardiac markers like CK and CK-MB in cases of Acute Myocardial Infarction. From the study it is concluded that the raised homocysteine, CK, CK-MB levels and raised lipids other than HDL-C are definitive risk factors for Ischemic heart disease.

Key words: Coronary Artery Disease, Creatine Kinase, Homocysteine, IHD, Lipid profile, Novel risk factors.

Introduction:

Ischemic Heart Disease (IHD) is one of the commonest causes of morbidity and mortality worldwide resulting in number of premature deaths. It poses a huge burden over community in terms social, economic and psychological fronts. The term 'Ischemic Heart Disease' is used to denote a situation where in the heart will not be in a position to satisfy the oxygen needs of the tissues as itself will be deprived of the oxygen. It is estimated that about two-thirds of the global estimated 14.3 million annual cardiovascular disease deaths occur in the developing world. By the year 2015, cardiovascular diseases could be the most important cause of mortality in India. The prevalence of coronary artery disease in India increased from 1% in 1960 to 9.7% in 1995 in urban populations, and in rural populations it has almost doubled in the last decade

[1]. Myocardial infarction (MI) represents the most important form of Ischemic Heart Disease where in there is a reduction in coronary blood flow due to coronary arterial obstruction. Thus Ischemic Heart Disease is often termed as coronary artery disease (CAD) or Coronary Heart Disease (CHD).The Prospective Cardiovascular Munster (PROCAM) [2]. simple scoring scheme postulates that 1) Advancing Age 2) Family history of premature myocardial infarction, 3) Diabetes Mellitus, 4) Systolic Blood Pressure, 5) Smoking, 6) Increase LDL-Cholesterol levels 7) Decreased HDL-Cholesterol and 8) hypertriglyceridemia constitute for the classical risk factors for the coronary artery disease. Despite the lack 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 classical risk factors. These newer risk factors, otherwise called as 'Novel risk factors' which includes 1.Lipoprotein-a, 2.Homocysteine, 3.Fibrinogen, and 4. h_scRP (C- reactive protein). Role of all the above risk factors in the causation of IHD is well established by numerous studies all over the world. Coronary Artery Disease in Indians is often premature and follows a malignant course and it is observed that incidence of IHD in young individuals who are less than 45 years of age is progressively increasing year by year. Hyperhomocystinemia is considered as independent risk factor for ischemic heart disease [3]. Owing to the burden caused by the IHD over the community it is imperative task for the healthcare professionals as well as Governments to diagnose and treat it well in advance so as to curb its incidence in the community for which identification and measurement of risk factors and biochemical markers for cardiac diseases such as Creatine Kinase (CK) is a must. Early measurement of biomarkers will help the treating physician to minimize the complications that arises due to progression the disease.

Aims and Objectives

The current study is aimed at determining the relationship between total plasma homocysteine as an independent causative agent of ischemic heart disease in young patients, to study the changes in the serum lipid profile and also of serum cardiac markers like CK and CK-MB in cases of Acute Myocardial Infarction. The important objective of the study being early detection, prompt intervention and prevention of complications of IHD in the community especially in the younger age group.

Materials and Methods

The current prospective study is conducted at Government Medical College and Government General Hospital Anantapuramu over a period of one year from January 2013 to December 2013 and a total number of 40 subjects (20 study group and 20 control group) who were less than or equal to 45 years of ages admitted in the Department of General Medicine with myocardial infarction. The patient details were collected in pretested Proforma, sample collection and estimations of various biochemical parameters were done using standard testing protocols, the results were tabulated in Microsoft Excel sheets and were analyzed using appropriate statistical methods.

The following parameters with the mentioned normal reference values were taken for the present study.

1. Total serum Creatine Kinase (CK): Estimated by Modified IFCC method.

a. Serum (Male):24 – 195 U/L at 37^0C
b. (Female):24 – 170 U/L at 37^0C

2. Serum Creatine Kinase (CK-MB): Estimated by Immuno-inhibition method.

a. CK-MB : >24 U/L at 37^0C
b. CK-MB to total Creatine Kinase: 6 % - 25 %

3. Lipid profile: Estimated by Enzymatic Method

a. Total Cholesterol: 150-250 mg%
b. Phospholipids : 125-272 mg%
c. Triacylglycerol : 30-180 mg%
d. Free fatty acids : 6-1 mg %

4. Plasma Homocysteine: Estimated by Homocysteine competitive immunoassay.

a. Normal fasting levels: 5 to 15 mmol/L.
b. Higher fasting levels : 15 to 30 mmol/L(Moderate)
c. Intermediate : 30 to 100 mmol/L and
d. Severe : >100mmol/L (Hyperhomocystinemia)

5. Criteria taken for Diagnosing Myocardial Infarction in this study were

a) A history of ischemic type chest discomfort.
b) Evolutionary changes on serially obtained ECG tracings.
c) A rise and fall in serum cardiac markers.

Results:

Estimated values of Serum enzymes viz., CK and CK-MB in 20 control subjects (Table No.1) revealed that mean and Standard Deviation (S.D.) of CK and CK-MB are 106.25 ± 9 and 17.9 ± 3.28 respectively. It is evident from the table that the levels of CK and CK-MB are within the normal

range in control Subjects. It is observed from the table that the mean and S.D. and p values of CK are 363.7 ± 94.94 and <0.01 respectively. It is evident from the table that there is an increase in the levels of CK in all myocardial patients and p-value is significant. The mean S.D., p- values of CK-MB are 41.5 ± 5.96 and p-values <0.001 respectively. Here the p-value is more significant.

Table 1: Levels of Serum Enzymes in Normal Subjects and Subjects with M.I.

S.No.	Normal Subjects		Subjects with M.I	
N=20	CK(units/L)	CK-MB(Units)	CK(units/L)	CK-MB(Units/L)
1	150	20	310	38
2	160	15	268	30
3	125	22	292	36
4	110	20	358	43
5	100	21	340	42
6	90	18	284	39
7	80	20	410	42
8	70	18	502	54
9	65	15	349	40
10	120	12	410	51
11	150	14	352	50
12	100	16	329	39
13	110	19	294	40
14	109	20	318	36
15	106	22	349	39
16	120	20	510	45
17	96	20	486	50
18	84	19	358	42
19	80	18	340	38
20	100	10	415	36
TOTAL	2125	359	7274	830
Mean	106.25	17.9	363.7	41.5
± SD	9	3.28	94.94	5.969
Est. Sed			21.229	15.458
t			3.276	15.458
p			<0.01	<0.001

Analysis of Lipid profile levels in normal subjects (Table 2) indicate that mean and S.D. of total Cholesterol, Triacylglycerol, HDL Cholesterol, LDL-Cholesterol and VLDL-Cholesterol are 188 ± 38.78, 132 ± 18, 42.6 ± 6.8, 1177 ± 8.87, 26.5 ± 3.61 respectively. So it can be inferred that the levels of Cholesterol, Triacylglycerol, HDL-C, LDL-C, VLDL-C are within the normal range in controls.

Table 2: Levels of serum Lipids in control Subjects (Units: mg/dL)

S.No.(N=20)	T.CHOL	TG	HDL-C	LDL-C	VLDL-C
1	242	98	38	184.4	19.6
2	158	140	52	78	28
3	143	153	38	74.4	30.6
4	228	140	33	167	28
5	240	132	48	117.6	26.4
6	182	129	45	111	25.8
7	246	124	49	182.2	24.8
8	199	148	44	125.4	29.6
9	171	137	49	95	27.4
10	160	124	42	93	24.8
11	168	150	55	83	30
12	138	127	33	79.6	25.4
13	151	89	38	95.2	17.8
14	151	148	52	69.4	29.6
15	212	102	32	159.6	20.4
16	243	150	48	165	30
17	145	133	45	73.4	26.6
18	157	142	39	89.6	28.4
19	226	147	36	160	29.4
20	202	141	46	127.8	28.2
TOTAL	3762	2654	862	2330.6	530.8
Mean	188	132	42.6	117	26.54
± S.D.	38.74	18.1	6.8	8.87	3.618

On analysis of lipid profile in subjects with myocardial infarction **(Table 3),** the mean, S.D. and p-values of total cholesterol was 293 ± 27.78 and <0.001 respectively. The mean S.D. and P-values of HDL-Cholesterol were 31.25 ± 6.16 and <0.001 respectively. The mean S.D. and p-values of LDL-C 216.8 ± 9.18 and p-values is <0.001 respectively. All these values are statistically significant. It is evident from the table that there is a rise in the total cholesterol, serum triglycerides, LDL-C, VLDL-C and fall in HDL-C levels in cases with Myocardial Infarction.

Table 3: Levels of Serum Lipids in subjects with M. Infarction (Units: mg/dL)

S.No.	T.CHOL	TG	HDL-C	LDL-C	VLDL-C
1	275	180	25	214	36
2	260	320	29	167	64
3	286	200	32	214	40
4	380	185	25	318	37
5	286	182	29	221	36
6	274	200	28	206	40
7	314	200	20	254	40
8	315	272	32	229	54.4
9	308	188	37	233	37.6
10	275	396	32	285	79.2
11	288	200	34	126	40
12	276	200	42	118	40
13	266	183	40	189	36.6
14	300	180	35	229	36
15	300	150	26	244	30
16	265	193	25	201	38.6
17	282	210	28	212	42
18	275	300	30	185	60
19	326	180	34	256	36
20	310	160	42	236	32
TOTAL	**5861**	**4279**	**625**	**4337**	**855.4**
Mean	**293**	**214**	**31.25**	**216.8**	**42.79**
± S.D.	**27.78**	**60.93**	**6.16**	**9.187**	**12.16**
Est. Sed.	**10.659**	**14.21**	**2.058**	**12.77**	**2.838**
t	**9.85**	**5.77**	**5.515**	**7.83**	**10.08**
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

When homocysteine levels of normal persons when compared with persons suffering with Myocardial Infarction, The levels of Homocysteine were raised significantly ($p < 0.001$) in the plasma of CAD patients (Table 4).

Table 4: Levels of Serum Homocysteine in Normal and M.I Groups

S.No.	Serum Homocysteine Levels	
N=20	**Control subjects (µ mol/L)**	**Subjects with M.I.(µ mol/L)**
1	6.8	21.5
2	8.2	23
3	7.8	22.5
4	6.2	21.2
5	8.1	22.4
6	7.9	22.6
7	7.8	21.8
8	8	23.4
9	8.1	22.8
10	7.6	22.2
11	8.1	21.9
12	7.8	23.4
13	6.9	21.6
14	8.2	23.2
15	6.4	24.6
16	6.9	21.1
17	6.6	21.9
18	5.2	22.2
19	5.6	23.8
20	6.2	21.2
TOTAL	**144.4**	**448.3**
Mean	**7.22**	**22.415**
± S.D.	**0.937**	**0.944**

Discussion

Coronary Artery Disease resulting from atherosclerosis and thrombosis (athero-thrombosis) is the leading cause of morbidity and morbidity in the community both in India and Abroad. Numbers of genetic and environmental factors are attributed to the cause of Coronary Artery Disease. Dyslipidemia, hypertension, diabetes and obesity are the genetic components of causation while, life style behaviors such as smoking, dietary habits and lack of physical activity are modifiable or environmental factors.

Results of this study indicate that there is a significant change in the values of lipid profile in the subjects suffering with AMI when compared to normal individuals. These findings are in agreement with many studies conducted over Indian as well as Western population and indicate that there is a consistent and strong positive relationship between plasma total cholesterol and incidence of Ischemic Heart disease.

Both primary and secondary trails have shown the possibility of reduction in cardiovascular morbidity and mortality by revascularizations along with addition to lipid lowering agents (statin group of drugs).

There were low levels HDL-C in AMI cases with significance of $p < 0.05$ indicates the inverse relation between the HDL-C and Adverse cardiac events. This finding is consistent with Framingham heart study **[4]**. However, the protective nature of HDL-C is poorly understood.

Our current study revealed increased LDL-Cholesterol levels significantly over control group. Raised LDL-C has been recognized as a independent risk factor by National Cholesterol Education Programme (NCEP) [5] in USA in 1988 and reaffirmed in 1993. The increased levels of LDL in turn lead to increased levels of oxidized LDL which contribute for the atherogenicity in the coronary vessels. Oxidized LDL possesses chemo-tactic and cytotoxic effects which result in the formation of foam cells that play a major role in the phenomenon of atherosclerosis.

Increased production of apolipoprotein B (genetic) by the liver leads to over production of VLDL followed by over production of LDL. Because of the increased levels of VLDL, there is increased transfer of triglyceride from VLDL to LDL in exchange for cholesterol esters. The triglycerides enriched LDL undergoes hydrolysis, thereby producing small dense LDL. It has been suggested that small dense LDL particles are more atherogenic because of greater arterial wall retention and increased susceptibility to oxidation.

Hypertriglyceridemia is prone to result in increased risk of Coronary Artery Disease. Increased triglyceridemia reflects increased VLDL-Cholesterol. Our study reiterated this fact as there were significantly increased levels of triglycerides in the subjects with acute myocardial infarction when compared to control group. There is some evidence that increased triglycerides cause atherogenicity, independent of LDL-Cholesterol. The mechanism of atherogenicity due to increased levels of triglycerides is attributed to the oxidized triglycerides lipoprotein. VLDL form an effective substrate for free radical reaction and the products could accelerate the progression of disease.

Total cholesterol to HDL cholesterol ratio was significantly high in Acute Myocardial Infarction group having about 74% of higher valve of mean and statistically highly significant. Total cholesterol to HDL-Cholesterol ratio (> 14.5%) is considered the most powerful predictor of coronary artery disease. It is prevalent in 42% urban Indian hypertensive subjects.

In Myocardial Infarction if the ischemia persists for long time, it causes severe damage to the heart muscle. Estimation of serum enzymes like CK, CK-MB is helpful in achieving diagnosis, in the assessment of therapy being given and to predict the prognosis of acute myocardial infarction cases. Serum enzymes are released in large quantities into the blood from necrosed heart muscle following myocardial infarction. The MB isoenzyme of CK has advantage over CK and LDH as it is not present in significant concentrations in extra cardiac tissue and therefore is more specific CK values are very useful to detect early cases. Total Creatine phosphokinase shows an increase following myocardial infarction in which it is increased earlier than other enzymes, beginning at 4- 6 hours, peaking on an average at 24 hours and returning to normal within 2 – 3 days. The area under the peak and slope of the initial rise are proportional to the size of the infarct. For accurate diagnosis of myocardial infarction Creatine-Kinase-MB activity should be measured. If the total Creatine phosphokinase activity is raised and Creatine Kinase-MB contributes more than 6% of the activity then myocardial infarction is considered highly probable. The estimation of serum total CK on the first day of the attack was determined and found that there was an increase in the levels of the CK in all the 20 cases of Myocardial Infarction against the controls. The extent of rise of total CK is an indicative of severity of the attack i.e., if the attack is severe the rise of CK is very high. Estimation of CK immediately after the attack is important diagnostic aid in the diagnosis of myocardial-infarction. The values of CK-MB were elevated within 4–6 hours after the onset of myocardial infarction and from this it can be inferred that elevated CK-MB values are highly diagnostic of myocardial infarction. The present study clearly shows that CK-MB activity is more than 6% total CK. These observations correlate with the findings of studies of Robert R. Esler et al [6] and John. J. Fenton et al [7].

Homocysteine, a sulphur containing amino acid, which is derived from the dietary methionine, has been associated with adverse cardiovascular events. Several studies show that the sites of adverse effect of homocysteine include endothelial surface, vascular smooth muscle cells, connective tissue, interaction with plasma lipoproteins, clotting factor and platelets. Homocysteine has been recently identified as a novel risk factor for coronary artery disease. It was in 1969; Mc Cully [8] made the first correlation between high plasma homocysteine and vascular disease. Later in 1976, Wilcken et al [9] published the first report that patients with coronary artery disease have abnormal homocysteine metabolism. Later, after 1990 many publications implicated elevated plasma homocysteine as an independent risk factor. In 1992 the first ever-positive prospective study was made on plasma homocysteine as a risk of myocardial infarction among the physicians in United States. Another

study in UK established that the plasma homocysteine concentrations were higher among Asian Indians and higher fasting homocysteine levels increased the mortality of the patients. Recently Ayav et al [10] reported that methylene tetrahydrofolate reductase (MTHFR) gene mutation causing Hyperhomocystinemia as a risk factor for CAD. Current study shows mean homocysteine levels in cases with myocardial infarction (22.41 ± 0.944, n=20) were significantly higher than the controls (7.22 ± 0.937, n=20) with P value of < 0.001 which is statistically significant. In conclusion my data suggests that plasma homocysteine levels were increased significantly in CAD patients and also that the homocysteine is the best predictor of CAD risk amongst other conventional risk factors associated with Coronary Artery Disease.

Conclusion

After the current study we believe that identification of major risk factors and effective control of these through population based strategies of prevention can decline the mortality and morbidity related to Ischemic Heart Disease. Estimation of CK, CK-MB, Serum Homocysteine and Lipid profile in suspected as well as in symptomatic cases will provide an effective diagnostic tool for early detection of IHD and ensures for its prompt management which ultimately result in decrease of mortality and morbidity related to IHD. As Myocardial Infarction will be the major component of CAD, it requires early diagnosis, prompt management and effective follow up so as to curtail its incidence.

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