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Study of Trace Element Status and Oxidative Stress in Acute Myocardial Infarction

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Abstract: *Coronary Artery Disease (CAD) is a major cause of morbidity and mortality affecting mostly young and middle aged population. Acute Myocardial Infarction (AMI) is an entity of CAD which is defined as a part of Acute Coronary Syndrome consisting of chest pain, dyspnoea. Recently it has been observed that trace elements and free radicals may play an important role in the development of AMI. The aim of the study was to estimate the levels of trace elements like serum copper, iron and zinc and oxidative stress in patients of AMI and to compare with the healthy controls and to find out the correlation between atherogenic index (AI), cardiac risk ratio (CRR) with malondialdehyde(MDA), taken as a marker of oxidative stress and trace element levels. The study was conducted taking 50 numbers of cases of AMI and an equal number of age and sex matched healthy controls. The results showed increase levels of iron and copper with (P<0.005) and decrease level of zinc with (P<0.005) in AMI patients which are found to be statistically significant. Also we found a strong positive correlation between serum copper, iron and MDA with AI and CRR. Serum zinc levels showed a negative correlation with AI and CRR indicating its protective effect. Thus trace elements may play a vital role in the causation and prevention of of AMI.*

Keywords: *AMI, Zinc, Copper, Iron, Oxidative stress*

I. Introduction

Coronary Heart Disease has been defined as an impairment of heart function due to inadequate blood flow to the heart compared to its needs caused by obstructive changes in the coronary circulation.[1] It is a major cause of morbidity and mortality contributing to 20-25% deaths in the developed world affecting mostly young and middle aged (30-69 yrs) population.[2] But recent studies shows that Coronary Artery Disease occurs a decade earlier in the developing country like India as compared to same age group of patients in the developed countries.

Acute Myocardial Infarction (AMI) is an entity of coronary heart disease which is defined as a part of acute coronary syndrome consisting of chest pain , dyspnoea with rise and fall in troponins or creatine kinase MB to values greater than 99 % of normal reference population.[3]

The contributing factors for the growing burden of AMI are increasing prevalence of cardiovascular risk factors especially hypertension, dyslipidemia, diabetes mellitus, obesity, smoking and physical inactivity.[4,5] Other factors such as micronutrients may be involved in etiopathogenesis.

In the development of AMI and evolution of atheroma (atherogenesis) emphasis is given on oxidative stress and damage caused by it. Trace elements and free radicals may play an important role in pathogenesis of atherosclerosis and also affect the lipid profile in AMI.[6]

All the trace elements are harmful to our body beyond a certain level. Copper and Iron are powerful promoters of free radical formation, thereby accelerating lipid peroxidation.[7] Zinc on the other hand acts as a biological antioxidant, which prevents lipid peroxidation and stabilizes membranes.[8]

In some studies it was found that trace elements may play a vital role, resulting in either harmful or beneficial effects by damaging or protecting vessel wall and altering lipid profile.

1.1. Aims and Objective:

In this context the purpose of study is to determine serum levels of copper, iron and zinc and oxidative stress in patients with AMI and control subjects who are age and sex matched and to find the correlation between Cadiac Risk Ratio (CRR), Atherogenic Index (AI) with the levels of copper, iron and zinc.

II. Materials and Methods

2.1. Study design and Patient:

Approval from the ethical committee was obtained and informed consent was obtained from individuals.

The present study was undertaken in the department of Biochemistry, V.S.S. Medical College and Hospital, Burla on diagnosed cases of AMI admitted to department of Cardiology and Medicine.The study included 50 numbers of AMI patients as cases and 50 numbers of age and sex matched healthy individuals as controls.

All the anthropometric parameters were recorded and a structured questionnaire was used to obtain data on family h/o diabetes mellitus, hypertension, past and present illness, dietary pattern, addiction and medication.

2.2. Study Group:

2.2.1. Inclusion Criteria:

- Age – 30-50 years
- AMI was diagnosed according to ECG finding and /or raised CK-MB isoenzyme or troponins)

2.2.2 Exclusion Criteria:

- Diabetes Mellitus
- Hypertension
- Nephropathy
- Pregnancy
- Person taking drugs containing copper, iron, zinc and diuretics.

2.3. Sample collection and preparation:

After an overnight fast, 5ml fasting venous blood was collected for following tests.

2.4. Biochemical investigations:

2.4.1. Routine blood tests:

- Fasting Plasma Glucose: Glucose oxidase – peroxidase method.
- Serum urea, creatinine, protein and albumin, Lipid Profile: total cholesterol, triglycerides, HDL, LDL and VLDL- calculated by Friedwald's formula.
- Urine examination: Routine and microscopic examination of urine.

2.4.2. Special tests:

- Serum zinc - By spectrophotometric method using nitro PAPS-..(2-(5-Nitro-2-pyridylazo)-5-(N-Propyl-N-Sulphopropylamino) Phenol) [9]
- Serum copper- By spectrophotometric method using Di-Br-PAESA.(4-(3,5Dibromo-2-pyridylazo)-N-Ethyl-N-(3-Sulphopropyl)Aniline.[10]
- Serum iron- By spectrophotometric method using Ferrozine.(3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine).[11]
- Serum malondialdehyde (MDA) – By Kei Satoh's thiobarbituric acid method.[12]
- Calculated parameters: Atherogenic Index- log(TG/HDL) and Cardiac Risk Ratio- TC/HDL

2.5. Statistical analysis:

- The significance of difference between two groups was tested using students t-test.
- Association between variables was determined using the Pearson's correlation analysis.
- Values of different parameters presented as mean ± SD.
- A p value < 0.05 was considered statistically significant.

III. Observation

3.1. Anthropological Measurement

Table 1

Parameters	Controls(n=50)		Cases (n=50)		'p' value
	Mean±SD	Range	Mean±SD	Range	
AGE(Years)	42.6±4.96	32-50	42.5±5.44	30-50	>0.05
AC (inch)	34.1±1.3	32-36	36.2±2.4	32-44	<0.0001
BMI (Kg/m^2)	21.7±1.5	19.2-23.8	29.6±1.8	25.8-32.2	<0.0001
SBP(mm Hg)	120±10.8	90-136	144±13.2	110-162	<0.0001
DBP(mm Hg)	80±6.6	70-88	98 ± 7.7	80-110	<0.0001

3.2.: Comparison of Serum Levels of Copper, Iron, Zinc and MDA In Study Groups:

Table 2

Parameters (µg/dl)	Controls (n=50)		Cases (n=50)		'p' value
	Mean ± SD	Range	Mean ± SD	Range	
COPPER	97.1±10.9	76-130	177±17.3	145-210	<0.0001
IRON	100±16.4	64-136	190±20.4	160-275	<0.0001
ZINC	87.8±8.33	74-105	62.1±6.06	46-74	<0.0001
MDA (micromol/l)	4.34±0.25	3.0-3.7	6.34±0.45	5.25-6.75	<0.0001

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3.3Routine Biochemical Investigations: Table 3

Parameters	**Controls**		**Cases**		**'p' VALUE**
	Mean ±SD	Range	Mean± SD	Range	
FBS (mg/dl)	86.4 ± 8.21	69 -104	84.6 ± 8.63	70 -100	>0.05
UREA (mg/dl)	33.9 ± 5.51	22 - 44	34.5 ± 7.65	18 - 48	>0.05
CREATININE(mg/dl)	0.82 ± 0.19	0.5 -1.3	0.84 ± 0.22	0.5 -1.3	>0.05
PROTEIN (gm/dl)	6.7 ± 0.37	6 -7.6	6.8 ± 0.47	6 -7.8	>0.05
ALBUMIN (gm/dl)	4.8 ± 0.26	4.2 – 5.3	4.6 ± 0.31	4.1 – 5.2	>0.05
TC (mg/dl)	155 ± 12.0	128 - 175	196 ± 19.0	168 - 240	<0.0001
TG (mg/dl)	142 ± 11.4	120 - 160	199 ± 22.8	180 - 280	<0.0001
HDL-C (mg/dl)	41.1 ± 4.68	30 - 50	30.4 ± 4.09	22 – 38	<0.0001
LDL-C (mg/dl)	89.5 ± 14.3	55 - 110	126 ± 18.1	96 -174	<0.001
VLDL-C (mg/dl)	28.4 ± 2.28	24 - 32	39.7± 4.13	36 - 56	<0.0001

3.4. Comparison of Atherogenic Index (AI) and Cardiac Risk Ratio (CRR) in Study Groups:

Table 4

Parameters	**Controls (n=50)**		**Cases (n=50)**		**'p' value**
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	
AI	0.53±0.08	0.42-0.65	0.81±0.12	0.67-1.0	<0.0001
CRR	3.8±0.60	2.9-5.1	6.58±1.32	4.6-9.5	<0.0001

3.5. Correlation of serum Copper, Iron, Zinc with MDA, Atherogenic Index and Cardiac Risk Ratio:

Table 5

	COPPER	**IRON**	**ZINC**
MDA	+ 0.315	+0.289	-0.022
AI	+ 0.719	+ 0.616	-0.025
CRR	+ 0.704	+ 0.540	-0.063

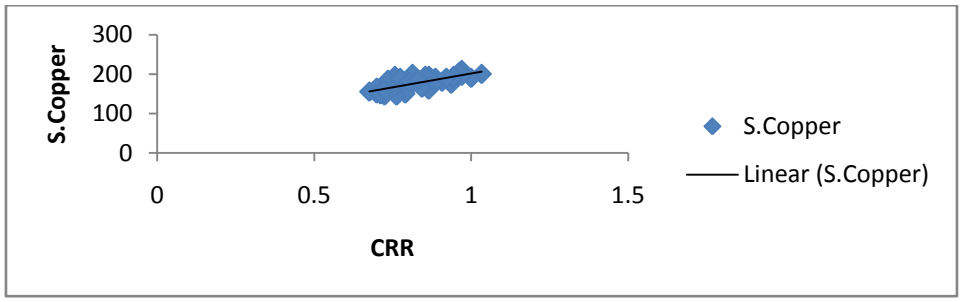

Fig 1: Graph showing correlation between Serum copper and cardiac risk Ratio

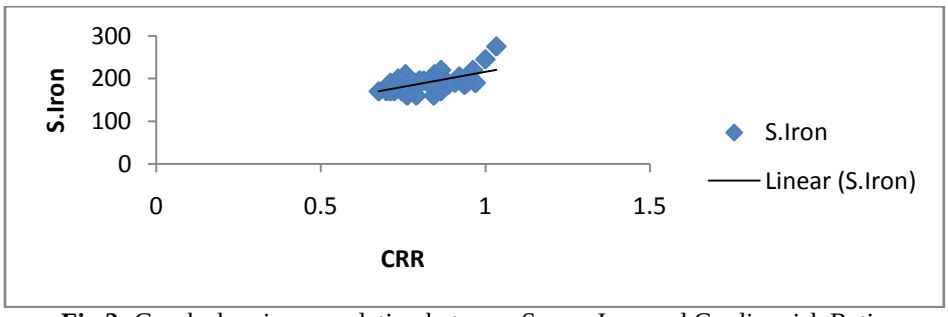

Fig 2: Graph showing correlation between Serum Iron and Cardiac risk Ratio

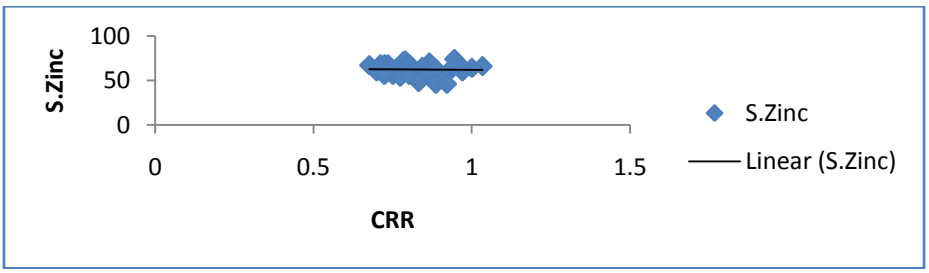

Fig 3: Graph showing correlation between Serum Zinc and Cardiac risk Ratio

IV. Discussion

Dietary trace elements are shown to be closely associated with heart disease risk as they are important constituents of various metalloenzymes which are responsible for the maintenance of myocardial integrity. Unbalanced serum concentrations of metals have been assumed to share in the causes of AMI.[13].

In the present study, the mean age of the cases was 42.5 year and that of controls was 42.6 years out of which males constituted 65% and females constituted 35%.The maximum number of cases were that of STEMI 64% followed by NSTEMI 36%.

BMI for controls was 21.7±1.5 Kg/m^2 and for cases it was significantly higher 29.6±1.8 kg/m^2 ($p<0.0001$). AC for control group was 34.1±1.3 inch and for cases it was 36.2±2.4 inch ($p<0.0001$) which shows the strong association of abdominal obesity with CAD.

Fasting blood sugar, serum urea, creatinine, protein and albumin didn't show any significant rise in cases as against controls as diabetics, hypertensives and renal failure cases were excluded in our study.

In the cases mean serum level of cholesterol and Triglycerides were 196±19 mg/dl and 199±22.8 mg/dl, which were significantly higher than those of controls 155±12mg and 142±11.8 mg/dl respectively ($p<0.0001$).The association of hypertriglyceridemia and coronary artery disease has been well documented by number of workers.[14,15] Triglyceride level may be independent of cholesterol level.[15]

The mean value of serum LDL-C was 126±18.1 mg/dl in cases which was significantly higher than controls 85.9±14.3 mg/dl ($p<0.0001$).It is now established that the relation of serum total cholesterol to atherosclerosis and CAD is mainly due to atherogenic effect of LDL-C; as 60-70% of plasma cholesterol is carried in LDL.[16]

Serum HDL-C in cases was 30.4±4.09 mg/dl where as in controls it was 41.1±4.68 mg/dl (p<0.001). HDL-C has been reported as a protective factor against atherosclerosis and responsible for centripetal transport of cholesterol from peripheral tissues including arterial wall to the liver. [17,18,19]

The mean value of serum $VLDL_C$ in cases was 39.7±4.13 mg/dl where as in control the level was 28.4±2.28 mg/dl (p<0.0001). High value of $VLDL_C$ were noticed in the patients with hypertriglyceridemia.[20]

The mean Atherogenic Index (LOG_{10} TG/HDL-C) of cases was 0.81±0.12 whereas in controls it was 0.53±0.08 (p<0.001) and the mean Cardiac Risk Ratio (TC/HDL) of cases was 6.58±1.32 whereas in controls it was 3.8±0.60. (p<0.001).

The serum level of copper was significantly higher in cases 177±17.3 µg/dl compared to controls with 97±10.9 µg/dl (p<0.0001). Increased serum copper levels are a part of a specific defense mechanism to provide more copper at the site of infarction to reduce its size and the extent of damage.[21] Also, the increase of ceruloplasmin, which is a copper containing enzyme and acute phase reactant, may account for the significant increase in serum copper levels.**[22]** Ceruloplasmin is an important intravascular antioxidant and protects tunica intima against free radical injury, which is the basis of constantly observed sudden increase in serum copper and ceruloplasmin levels.**[23]**

In controls the mean iron was 100±16.4 µg/dl while in cases it was significantly higher with a mean value 190±20.4 µg/dl (p<0.0001). Iron induced oxidative stress in the form of increased generation of reactive oxygen species, in a series of Fenton like reactions [24], makes the vascular endothelium vulnerable to dysfunctional injury, leading to increased susceptibility to AMI. Moreover, total serum iron in acute myocardial infarction correlated positively with serum ferritin level, which suggest an acute reaction response to inflammation and with the more availability of free iron on account of declined total iron binding capacity, the formation of ferritin gets markedly induced in a protective, body defense measure to sequester the free iron along with its known anti-oxidative properties (Crichton et al., 2002).[25] It is the free ionic form of iron that is harmful due to its pro-oxidative properties, which generates reactive free radicals.[24] Superoxide produced during oxidative stress can mobilize free catalytic iron from ferritin (Halliwell, 1994)[26] and facilitate the formation of Hydroxyl ion. Reactive oxygen species, superoxide cause lipid peroxidation and endothelial dysfunction in vessels (Salonen et al., 1994).[27]

In controls the mean zinc was 87.8±8.33 µg/dl and for cases mean was 62.1±6.06 µg/dl. The serum level of zinc was significantly lower in cases (p<0.0001). Low serum Zn levels in patients have been related to excess release of steroids due to the release of leukocyte endogenous mediators which redistribute the body Zn from serum and may cause a drop in serum Zn and also due to elevated levels of α2-macroglobulin which is a transport protein containing large amounts of Zn.**[28]** Induction of metallothionein by zinc has been shown to alter the physiological disposition of copper**[29]** and metallothionein has a greater binding capacity for copper than for zinc**[30]**, so causing elevation of serum copper level and lowering serum zinc level. Zinc can compete with iron, by Fenton reaction, in production of free radicals and can "interfere with metal catalyzed lipid peroxidation". Zinc diminishes the weakening effect on the membrane caused by the peroxidative damage.

In the present study, mean serum malondialdehyde(MDA) was 6.34±0.4 µg/l in cases and 4.34±0.25 µg/l in controls with a significant p value of <0.001.Guttridge[31] and Sushamakumari S. et al[32] showed that an alteration in the metabolism of lipid peroxides is closely and strongly associated with myocardial damage as indicated by increasing MDA in the heart tissue on induction of myocardial infarction. Reasons for this increased peroxidizability of damaged tissues, inactivation of some antioxidants and leakage of antioxidants from the cells and the release of metal ions (especially iron and copper) from storage sites and from metalloproteins hydrolyzed by enzymes released from damaged lysosomes. There occurs a direct relationship between lipid peroxidation and serum levels of copper, iron and inverse relationship with zinc. Increased lipid peroxidation leads to multiple membrane damage and disturbance in cardiac metabolism, structure and function. Findings of Comar CL and Chvapil M[33,34] support our result. Zinc can compete with iron, by Fenton reaction, in production of free radicals[35].Thus zinc can "interfere with metal catalyzed lipid peroxidation". Zinc diminishes the weakening effect on the membrane caused by the peroxidative damage. Significant negative correlation between MDA & zinc were found in AMI and positive correlation between MDA and iron and copper.[36]

Atherogenic Index and Cardiac Risk Ratio are positively correlated with MDA, serum copper and iron and negatively correlated with serum zinc. This suggests higher risk of CAD with increase in copper and iron levels whereas zinc has a protective effect.

V. Conclusion

It is concluded that nutritional disturbances of trace elements and antioxidants is to be considered as major risk factor for AMI patients. Our study supports the hypothesis that, low levels of zinc and increased levels of iron and copper catalyze the Fenton reaction in generation of free radicals, which in turn increase free

radical mediated oxidative stress. Our findings can help the clinicians in the determination of oxidative stress in AMI and advocate nutritional balance of trace elements in diet for prevention of CAD.

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