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Research Article

RELATION OF ANTIOXIDANT STATUS AND LIPOPROTEIN (a) LEVEL IN MYOCARDIAL INFARCTION PATIENTS : A CLINICAL STUDY TO EVALUATE THE ANTIOXIDANT/ LIPOPROTEIN (a) RATIO AS A NOVEL PARAMETER TO INDICATE CARDIOVASCULAR ACCIDENT

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ABSTRACT

Lipoprotein (a), a prominent marker of cardiovascular accident, was first described by Berg in 1963(3). Lipoprotein (a) [Lp(a)] has been considered as one of the distinguished signature in myocardial infarction and for many years.[1.] Owing to incomplete scientific evidence in clinical practice, screening for and treatment of high Lp(a) levels is primarily essential . On the other hand free radicals, now shown to be one of the underlying causes in neurological and immunological diseases, possess tremendous affinity to pair with electrons to form couples. These high velocity reactions have been shown to be the major mechanism behind the harmful effect of free radicals. The present study aims to correlate the antioxidant status ,which has been shown to be associated with kidney, heart and lung diseases, with lipoprotein (a) or Lp (a), The free radicals, basically species with unpaired electrons have been found to be of the causative agents for shifting of equilibrium of pro-oxidant antioxidant balance. **Aims and Objectives:** Aim of our study is **1.**To determine the level of Lp (a) and antioxidant status of patients viz. the antioxidant *in vivo* parameters like catalase [CAT], superoxide dismutase[SOD] and reduced sulfhydryl group[GSH] **2.** To find out the significance of relation between parameters of antioxidant and Lipoprotein (a) level among the patients of cardiovascular accident **3.** To compare the Lipoprotein (a) and antioxidant status **4.** To find out the significance of Lipoprotein (a) level antioxidant status and correlate the two parameters in acute myocardial infarction [AMI] patients. **Materials and Methods:** Fourty one (41) patients with acute myocardial infarction were selected from a series of consecutive patients admitted in the coronary care unit (CCU) of different medical colleges and /or hospitals or Nursing Homes or health care centres. Lp(a) was quantified by immunoturbidiometric method and other antioxidant parameters by spectrophotometric /enzymatic assays. **Results:** Serum LP(a) concentration in control group is 30.50 ± 25 mg/dl with maximum 134mg/dl . In the case group AMI patient group in both male & female patients with average mean LP(a) concentration is 78.95±45 mg/dl, the maximum value is 485 mg/ dl. There is direct correlation between antioxidant enzyme catalase and inverse relationship between superoxide dismutase and reduced sulfhydryl group [Equivalent to reduced glutathione][GSH] **Discussion:** There is significant difference between the two groups. ($p < 0.05$). Lp-(a) concentration level in patients with AMI is higher than the control group. An elevated Lp(a) concentration is associated AMI and a risk factor for AMI ., suggesting that Lp(a) may play an important role in the genesis of thrombotic coronary occlusion and the occurance of AMI. The relationship of antioxidant enzymes proves that the underlying causative agent of pathogenesis of cardiovascular accident is due to ROS[Reactive oxygen species]**Conclusion: I**t is suggested to make Lp(a) serum level determination test as a routine laboratory test for identification of risk factor for AMI .The antioxidant parameters can also be taken as accessory parameters in the detection of CAD.

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Keywords: LP(a), AMI , catalase, SOD, GSH

1. A) INTRODUCTION:

Lipoprotein (a) was first described by Berg in 1963(3). Lipoprotein (a) [Lp(a)] has been considered a cardiovascular risk factor for many years.[1.] Owing to incomplete scientific evidence, screening for and treatment of high Lp(a) levels have to date been performed principally by lipid specialists. However, during the last few years, major advances have been achieved. Lipoprotein (a) is a cholesterol–rich lipoprotein particle composed of an LDL particle and a large glycoprotein, apolipoprotein(a) [apo(a)]. It has been suggested that it is a coronary risk factor independent of increase in other serum lipids (eg. cholesterol and triglycerides), hypertension, smoking obesity and a family history of IHD (20). Free radical and antioxidant balance play a critical role in the pathophysiology of development of diseases including varied categories of vascular accidents. Catalase [CAT], superoxide dismutase [SOD] and reduced form of total sulfhydryl content as equivalent of the reduced glutathione [GSH]. Cardiovascular accident has been the single largest causative agent of death not only in the developed countries but also in the developing nations of the world. The structure of lipoprotein (a) is similar to plasminogen and tPA (tissue plasminogen activator) and it competes with plasminogen for its binding site, leading to reduced fibrinolysis. Lp(a) also carries cholesterol and thus contributes to atherosclerosis.(8, 18) In addition, Lp(a) transports the more atherogenic proinflammatory oxidized phospolipids which attract inflammatory cells to vessel walls(18,10)–and leads to smooth muscle cell proliferation.(20) Serum Lipoprotein(a) and disease: High Lp(a) in blood is a risk factor for coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke.[21] ·Lipoprotein(a) - Lp(a)[27] **Desirable**: < 14 mg/dL (< 35 nmol/l) **Borderline risk**: 14 - 30 mg/dL (35 - 75 nmol/l) .**High risk**: 31 - 50 mg/dL (75 - 125 nmol/l) .**Very high risk:** > 50 mg/dL (> 125 nmol/l) . The present study is aimed at elucidation of probable link between Lipoprotein a or Lp(a) as a direct indicator of cardiovascular accident and the causative possibility and the interrelationship between the pre mentioned antioxidant status and consequently the stature of devastation caused by free radical damage in clinical patients with and without confrontation with cardiovascular accident.Relating to the separate parameters viz. Lp(a), as indicator of cardiovascular accident, catalase, superoxide dismutase and reduced sulfhydryl group as the pointer for antioxidant status and the pro-oxidant antioxidant balance, a correlation is being investigated as po oxidant antioxidant status as a causative probability of cardiovascular accident.

B) Aims and Objectives:

i) Aim of our study is to determine the level of Lp (a) and enzyme levels of antioxidant status in patients with acute myocardial infarction

ii) To find out the correlation between the antioxidant status and of Lipoprotein (a) level among the patients of Acute Myocardial infarction (AMI).

iii) To compare the Lipoprotein (a) and enzymes of antioxidant status amongst the AMI patients

iv) To find out the significance of correlation between Lipoprotein (a) level and antioxidant status patients with cardiocascular accident.

C) Materials & Method:

Patients: 41 patients with acute myocardial infarction were selected from a series of patients admitted in the coronary care unit (CCU) of different hospitals and /or medical colleges had the complete data including family history, laboratory findings and clinical data.

All patients and control subjects were older than 35 years old. The healthy controls were selected from subjects who underwent routine laboratory examination for check up.

Inclusion criteria in this group were: absence of a history of smoking, cardiovascular disease and and the age over 35 years old. Fourty two subjects with these criteria were chosen.

Blood sampling and assay:

Fasting venous blood sample from all patients (the day after admission to CCU) and control subjects were collected. Blood was centrifuged for 10 minutes and the serum stored at -20°c until analyzed.

Lp(a) was quantified by immunoturbidiometric method (CRM Diagnostic system, imported and mnanufracturd by Sirus Biocare pvt. Ltd., p-25, Kalindi Housing Scheme, Kolkata-700089 west Bengal).

Total cholesterol, HDL –cholesterol and triglyceride were determined by enzymatic methods (by Beckman Coultier , Auto analyser Reagent.).
Friedewald formula was used to calculate the LDL – cholesterol level.
Samples with severe hemolysis or TG more than 2000 mg/dl, were excluded. The Lp(a) samples of patients and controls were unknown for technician who measured them

Estimation of Superoxide Dismutase (SOD) Activity: An Enzymatic Parameter for Antioxidant Status

Superoxide Dismutase activity was determined according to the method of McCord and Fridovich. The assay procedure involves the inhibition of epinephrine auto oxidation in an alkaline medium(pH10.2).The reaction was started by adding epinephrine solution to the assay mixture, containing tissue supernatant , sodium salt of EDTA and sodium carbonate and the change in the extinction coefficient was followed at 480nm in a spectrophotometer.

The rate of change of extinction, as reported, was of change of 0.025 per minute at 25 degree celcius.[The enzyme activity was expressed in arbitrary units considering 50% inhibition in the reaction mixture under the experimental conditions as on unit of SOD].

Estimation of Catalase Activity

Catalase activity was determined according to the method of Luck et al. 100 microlitre of serum was added to a solution of 3 ml of Hydrogen Peroxide in Phosphate buffer (50mM phosphate buffer, pH 7.0 and 30% Hydrogen Peroxide).The change in optical density at 240nm per unit time was a measure of catalase activity in a specific buffer – Hydrogen Peroxde medium.

Determination of Sulfhydryl Group content as Reduced Glutathione

The determination of total sulfhydryl group as equivalent to reduced glutathione content was done according to the method of Ellman .10 mM phosphate buffer (pH=8.0) was used as a medium. To this was added 0.2ml of 10mM DTNB[5,5'-dithiobis-(2-nitrobenzoic acid)] of pH 7.0 pepared in 10mM phosphate buffer[pH 7.0].The mixture was kept at room temperature for 20 minutes and the absorbance was read at 412 nm using 2 mM solution of [GSH] as standard.

Statistical Analysis: All biochemical and clinical data were recorded. He Lp(a) level of acute MI patients with those of age and sex–matched controls. Based on manufactures instructions, Lp(a) > 30 mg / dl is the threshold value linked to its pathologic effects. We define subjects with > 30mg / dl as those with high Lp(a) and patients with LP(a) level > 50 mg /dl as very high LP(a) and examined its frequency in acute myocardial infarction Continuous variables were reported as mean ±1 standard deviation.

Result:

Table 1: Summary of patients and control subjects datas

	Case(AMI Patient)	Control
Total number	41	46
Age (Mean)	51±3.42	55±2.41
Female /Male	12/29	11/35
T_2DM	14	M 06
Hypertension	27	12

Biochemical datas of serum LP(a) concentration in control group is (30.2+_ 2.5mg/dl with maximum 134mg/dl). In the case group (AMI Patient group both male& female patiens) average mean LP(a) concentration is 78.2+_5.9 mg/dl, with maximum value 485 mg/ dl. There

is significant difference between the two group. (p< 0.05).

Based on the datas and LP(a) reagent manufracturer (CRM Diagnostic) direction LP(a) serum level more than 30 mg/dl is considered as threshold value. Now in the case Group (AMI patients 36/42 = 85.71%) with high LP(a) level (LP(a) > 50 mg/ dl) and control group (39/42 =92.85%) with LP(a) level equal to 30.50+_ 25 mg/dl) are statistically significant . (p value < 0.05).

The mean LP(a) in women (AMI case Group, 98.7 mg/dl) that is higher than male ((AMI case Group, 59.2 mg/dl).

The female case group has very high LP(a) concentration (14/18= 77.77% , LP(a) level > 50 mg/ dl)) and male case group (10/24 = 23.80% , group has very high LP(a) level > 50 mg/ dl) .(range 19.1mg/dl to 438 mg/dl), which is statistically significant. (p value <0.01))

The LP (a) concentration level is independent of lipid profile in blood.

The mean total cholesterol, TG(triglyceride) , HDL –Cholesterol, LDL- Cholesterol, has been presented in table no .2

Table. 1 Demographic data of patients and controls

Table: 2 Summary of lipid profile in patients and controls.

	Case (AMI Patient)	Controls.
LP(a)	78.2 +_ 5.9*	30.2+_ 2.5
Total Cholesterol	180.4+_ 5.0**	160.5+_3.5
LDL-Cholesterol	105.1+_ 6.0*	95.4+_4.0
HDL-Chosterol	45.4+_1.2*	51.4+_1.1
TG (Triglyceride)	140.3+_8.0*	130.6+_7.7

*P<0.05
**p<0.10

Table 3: Catalase activity in Patients with Coronary Artery Disease

	Myocardial Infarction Patients	Normal
Mean Catalase Activity (Total)	36.23±0.52*	9.42±0.64*
Catalase Activity in Males	42.74±1.53**	9.46±2.26***
Catalase Activity in Females	33.46±2.64**	8.26±2.68***
Ratio of catalase Activity in Male /Female population	1.28	1.15

*P<0.01
**P<0.05
***p<0.1

Table 4: Activity of Superoxide Dismutase Level in patients with myocardial infarction

	Patients with coronary Artery disease	Control/normal
Total SOD[Superoxide Dismutase] Activity	74.26±12.31***	127.62±7.04**
SOD[Superoxide Dismutase Activity in Male]	72.68±7.42**	131.42±1 2.64*
SOD[Superoxide Dismutase] Activity in Females	84.62±11.48**	144.46±10.58*
SOD [Superoxide Dismutase] Activity in Male/Female Population	0.86	0.91

*P<0.01
**P<0.05
***p<0.1

Table 5: Total Sulfhydryl Content [Reduced] [Antioxidant Parameter] in Myocardial Infarction patients

	Patients with coronary Artery disease[µM/mL]	Control/normal [µM/mL]
Total Sulfhydryl Content [Reduced] [Antioxidant Parameter] X 10	3.46±0.26**	0.84±0.24***
Sulfhydryl Content [Reduced][Antioxidant Parameter] in Male X10	3.78±0.64**	0.82±0.42*
Sulfhydryl Content [Reduced][Antioxidant Parameter] in FemalesX10	2.92±0.42*	0.94±0.28**
Sulfhydryl Content [Reduced] in Male/Female X10 Population	1.30	0.87

*P<0.01
**P<0.05
***p<0.1

DISCUSSION:

In this study serum lipoprotein(a) LP-(a) concentration were compared with Acute Myocardial Infarction (AMI) patients and healthy normal subjects.

We showed that in average LP-(a) concentration level in patients with AMI is higher than the control group.

Another important findings are that LP(a) level in women patients are higher than male group patients. The LP-(a) level in blood is independent of lipid profile's of blood. In this study because less number tribal versus non-tribal case no statistical difference can be ascertained wkich will be followed in subsequent study.

There are few studies regarding LP-(a) level in AMI. In one Indian study Singh's et al, of in 1999 has opined that Lp(a) alone could correctly discriminate a CHD individual from a control subjects by 95%. Estimating of Lp(a) together with

albumin provided 99% correct discrimination between control and CHD patients..

David j. Moliterno et. al showed that elevated plasma concentration of LP-(a) are associated with coronary artery atherosclerosis in Caucasian. They also showed that African American have higher median plasma concentration than Caucasian but they do not have a greater incidence of coronary atherosclerosis. (7).

In a study by Abraham A. Ariyo it was shown that among oider in United states elevated lipoprotein (a) is an independent predictor of stroke., death from vascular disease and death from any cause in men but not in women. These data support the use of LP(a) levels in predicting these events in older men. (8)

Laron Z et al in their investigation determined the effect of Human Growth hormone (Hgh) and insulin like Growth factor –i(IGF-I) on circulating LP(a); Long term GH treatment increases and IGF- I decreases circulating leves of LP(a).it seems that LP(a) is specificallyan independent risk factor in diabetes.(11- 12).

Nogues X et al suggested a discriminant cut off of LP(a) concentration equal to 20 mg/dl or30mg/dl in enzyme immunoassay.(13).in the future there may be therapeutic method to reduce LP(a) levels which maybe proven to be useful in preventing myocardial infarction.

The antioxidant status showed the mean catalase activity in myocardial infarction patients was 36.23±0.52 in comparison to normal value of 9.42±0.64, which shows the excessive production of free radicals or reactive oxygen species in the system thus making overwhelming production of catalase, required for neutralization of free radicals by the following reaction: $H_2O_2 = H_2O + {}^1/_2O_2$

The increase in catalase activity was found to be higher in male population viz. 42.74±1.53 compared to females viz 33.46±2.64, reflecting the probability of necessity of higher concentration of reactive oxygen species(ROS) for induction of vascular incidence in males , than in females. Usually the rate of cornonary artery diseases is more prevalent among males than in females. So, it may be presumed that the reason for, in spite of the need of higher concentration of ROS required for cardiovascular accident in males, the rate of CAD is higher in males is due to manifold higher degree of stress and ROS instigating factors exposed in Indian scenario.

The activity of superoxide dismutase is 74.26±12.31 in patients with CAD compared to 127.62±7.04 in normal persons with no cardiovascular accident.The amount of SOD concentration is lower in male patients viz. 72.68± 7.42 compared to female patients viz. 84.62 ± 11.48, showing the higher percentage of extinction of ROS by antioxidants in male patients, resulting in higher exhaustion of *in vivo* antioxidants in males. The level of SOD in normal males viz. 131.42 ±2.64 is less than that of normal females, reflecting the level of neutralization of ROS is more in case of males.

In case of total sulfhydryl group content in reduced form [Equivalent to reduced Glutathione][GSH], the concentration of GSH is 3.46±0.26 in patients with CAD compared to normal patients with no cardiovascular accident is 0.84±0.24. The values of male patients are 3.78±0.64 compared to their normal counterparts estimated as 0.82±0.42. The values of females patients is 2.92±0.42 as compared to the corresponding normal persons viz.0.94±0.28.Both the values of SOD and glutathione[GSH] proves the role of free radicals or antioxidants in maintenance of non-pathological condition and formation of disease condition in case the pro oxidant antioxidant balance topples towards the free radical or pro- oxidant side.

The development of pathological syndroms due to pro oxidants have been mentioned earlier. That the underlying cause of Lipoprotein a , Lp(a) as the indicator for myocardial infarction has been documented. The direct relationship between Lp(a) and catalase and the inverse relationship between SOD , reduced sulfhydryl group and Lp(a) confirmed the role of free radicals in the pathogenesis of CAD. The study coincides with the evidence of antioxidant enzymes and redox regulating thiol protein in pathological conditions. In fact oxidant is involved in modulation of cell biochemical activities that play important roles in pthophysiology of diseases. Antioxidant enzymes and thiol proteins regulating cellular redox state constitute the major cellular protection against oxidants and their disbalance can be reasoned as to cause the cardiovascular accidents in these cases.The direct relationship of Lp(a) as marker of CAD is added with the antioxidant parameters of catalase, SOD and glutathione in reduced state or

reduced sulfhydrl group which can also be considered as supplementary parameters in CAD. There are direct scope of research involving the genes of antioxidant in relation to CAD and development of molecular or genetic medicine relating to Lp(a), and the antioxidant enzymes as applications in gene targeted medical sciences.

In another study Dumitrescu L, *et. al* Prior studies of the relationship between LP(a) and ethnicity have shown inconsistent results. Lipoprotein (a) levels seem to differ in different populations. For example, in some African populatation, Lp(a) levels are, on average higher, than other groups, so that using a risk threshold of 30 mg/dl would classify up to > 50% of the individuals as higher risk.[19][20][21][22] Some part of this complexity may be related to the different genetic factors involved in determining Lp(a) levels. One recent study showed that in different ethic groups, different genetic alterations were associated with increased Lp(a) levels.[23].In one South Indian study Rajasekhar et al on 2004.,(Better assessor of coronary heart disease in south Indian population) also suggested that Lp(a) level > 25mg/dl is risk factor for CHD.

In fact association between catalase gene polymorphism and risk of chronic Hepatitis B virus related liver cirrhosis and hepato cellular carcinoma has been established .In acute and chronic myeloid leukemia , catalase levels were found to be higher than the range for normal granulocytes, whereas in acute and chronic lymphatic leukemia, the levels were of the same order as thoses for normal lymphocytes.

E) Conclusion: An elevated Lp(a) concentration is associated AMI and a risk factor for AMI ., suggesting that Lp(a) may play an important role in the genesis of thrombotic coronary occlusion and the occurance of AMI. So it is suggested to make LP(a) serum level determination test as a routine laboratory test for identification of risk factor for AMI and to follow proper treatment to reduce LP(a) level in serum.

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