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ANTIOXIDANT STATUS AND SERUM LIPOPROTEIN(A) LEVEL IN CORONARY HEART DISEASES

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ABSTRACT

Coronary heart disease (CHD) has become the most important cause of premature morbidity and mortality. The chief risk factors for CHD includes Smoking, Hypertension, Diabetes mellitus, Hypercholesterolemia, Low high density lipoprotein, Obesity, Mental stress, Type A personality and Genetic factors. Lipoprotein (a) has been established as a strong independent risk factor for premature CHD, which is highly thrombogenic and antifibrinolytic which begins to block the arteries much earlier than other risk factors. Free radicals can cause metabolic disturbances and cell injury, damage cardiovascular system by atherosclerosis and ischaemia reperfusion. Increased oxidative stress may result from over production of precursors to reactive oxygen radicals and / or decreased efficiency of inhibitory and scavenger systems. Antioxidant functions as blockers of radical processes before they can damage various biomolecules or prevent oxidative damage from spreading out the effect. The present study comprises of 80 patients, 20 patients of myocardial infarction, 60 patients with risk factor of Coronary heart disease (CHD), including Hypertension, Diabetes mellitus, Smoking and Obesity. 20 healthy age and sex matched subjects served as controls. All the patients in the study group and control group were aged between 30-60 years. This study is planned to determine the antioxidant status and to estimate Serum lipoprotein (a) levels in patients of CHD and in patients with risk factors for CHD. Study comprised of estimation of Biochemical parameters like serum Lipoprotein(a), serum Malondialdehyde (M.D.A.), Blood Glutathione, Serum Vitamin E, Plasma Vitamin C and Plasma Vitamin A (special ones) with routine parameters like Blood Glucose, Serum Cholesterol, Serum Triglycerides, Serum HDL, Serum LDL, Serum VLDL, TC/HDL, LDL/HDL ratio.

KEY WORDS:- Coronary heart disease, Lipoprotein (a), MDA , Antioxidants.

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INTRODUCTION

Coronary heart disease has been defined as "the impairment of cardiac function due to inadequate blood flow to the heart compared to the metabolic needs, caused by occlusive changes in the coronary circulation to the heart". It is the condition of diverse etiologies all having a common disturbance of cardiac function due to imbalance between oxygen supply and demand. The spectrum ranges from an asymptomatic patient to sudden death, Commoner presentations being Angina Pectoris of effort, acute myocardial infarction, irregularities of the heart including Cardiac arrhythmias, Cardiac failure, and Ischemic cardiomyopathy Coronary heart disease (CHD) has become the most important cause of premature morbidity and mortality. With the advancement of medical science in the 20th century, there has been a remarkable increase in the life expectancy throughout the world by controlling infections and consequently more number of deaths are recorded due to degenerative causes like coronary atherosclerosis. CHD is very important medical problem and in spite of a large number of researches for diagnosis and treatment, it takes a huge toll of human lives all over the globe. CHD produces localized ischaemia of the myocardium and when the occlusion is complete, myocardial infarction takes place. There are various risk factors, presence of which makes person more prone to develop CHD, the risk factors for CHD includes Smoking, Hypertension, Diabetes mellitus, Hypercholesterolemia, Low high density lipoprotein, physical inactivity, Obesity, Mental stress, Type A personality and Genetic factors. Lipid peroxidation is a free radical mediated reaction, may play a significant role in the initiation and progression of atherosclerotic plaque[1.] It is not possible to measure free radicals, so lipid peroxidation is measured in terms of Malondialdehyde (MDA), which is end product of lipid peroxidation. There are natural protective molecule which scavenge free radicals called antioxidants e.g., Glutathione(GSH), Vitamin E, Vitamin C and vitamin A. Glutathione is a major antioxidant produced by the cell, protecting it from "free radicals". Endogenous cardiac GSH provides protection against myocardial dysfunction associated with short periods of ischemia – Recovery in glutathione-depleted hearts was improved when the reperfusate was supplemented with GSH[2.] The level of these antioxidants will determine the antioxidant status.

Lipoprotein (a) [Lp (a)] is a specific class of lipoprotein particles consists of apo (a) which is linked with apo B_{100} by a disulfide bond[3]. Lp (a) now been established as a strong independent risk factor for premature CHD, which is highly thrombogenic and antifibrinolytic, which begins to block the arteries much earlier than other risk factors. Lp (a) provides a carrier system for LDL-C and promotes cholesterol accumulation in cells. Oxidized LDL-C and Lp (a) accumulate in excessive amounts in macrophages ("foam cells") forming fatty streak. Intact Lp (a) deposition has been demonstrated in the arterial wall, and venous grafts and atherosclerotic plaques. A study of the atherectomy specimen showed a correlation of plaque alpha-actin and Lp (a), indicating a role of Lp(a) in plaque growth as well. Further, it stimulates smooth muscle cell proliferation avidly binds to arterial proteoglycans, and fibronectins. Plasma homocysteine increases affinity of Lp (a) for fibrin, thus increasing its atherogenic potential. Oxidized Lp(a) is also implicated in the causation of endothelium dysfunction. In induction of atherogenesis, recent evidence indicates that Lp(a) adhesion molecules-An endothelial cell - activating effect of Lp(a) is an potent surface expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) and E-selectin. This may be an important event in the initiation of atherogenic disease [4] .Endothelial cells due to surface-connected fibrinolytic system are important for fibrinolysis. Lp(a), because of its plasminogen like apo(a), interferes with fibrinolysis due to inhibition of plasminogen like apo(a), interferes with fibrinolysis due to inhibition of plasminogen like apo(a), interferes with fibrinolysis due to inhibition of

plasminogen binding to its high affinity sites. Lp(a), to some extent, regulates the synthesis of a major fibrinolytic protein, Plasminogen Activator Inhibitor-1(PAI-1). These prothrombotic events are now considered essential to the genesis of atherosclerosis [5]. The present study is planned to know the co-relation of total antioxidant status by the parameters mentioned above and concentration of Lp(a) and to evaluate of whether they can be used as monitoring indices in Coronary heart diseases.

MATERIALS AND METHODS

SELECTION OF GROUP

The present study comprises of 80 patients, 20 patients of myocardial infarction, 60 patients with risk factor of Coronary heart disease (CHD), including Hypertension, Diabetes mellitus, Smoking and Obesity, seeking medical care in Goa Medical college Hospital, Bambolim, Goa, during the period of November 2004 to October 2005. 20 healthy age and sex matched subjects served as controls. All the patients in the study group and control group were aged between 30-60 years. The consent was obtained from the institutional ethical committee and the patients consent for the tests was also obtained In both the groups, a detailed history was obtained and a thorough clinical examination was carried out as per the structured preformat.

Collection of Blood Samples

5ml of blood samples were collected in plain bulbs and were allowed to clot. After 1 hour, serum was separated by centrifuging at 2500 rpm for 5 minutes at room temperature. Serum was used for measurement of M.D.A, Vitamin E and Lp(a).

5ml of blood samples were collected in flouride bulb, plasma was separated and used for measurement of Vitamin C, Vitamin A and whole blood for measurement of blood Glutathione. All estimations were done within 24-48 hours after specimen collection.

ROUTINE BLOOD INVESTIGATIONS

1. Estimation of Blood Glucose was carried by Folin-Wu's method[6].
2. Estimation of Serum Cholesterol was carried by Libermann Burchard method[7]
3. Estimation of Serum Triglycerides was carried by GPO-PAP method[8]
4. Estimation of Serum HDL Cholesterol was carried by Phosphotungstate Magnesium method[9]
5. Estimation of Serum LDL and VLDL Cholesterol was calculated by Friedewald Formula[10]

SPECIAL BLOOD INVESTIGATIONS

ESTIMATION OF SERUM LIPOPROTEN(a)[11]

The measurement of Lp(a) is quantitative turbidimetric test. Latex particles coated with antibodies anti-Lp(a) are agglutinated when mixed with samples containing Lp(a). The agglutination causes an absorbance change, dependent upon Lp(a) contents of samples that can be quantified by comparison from a calibrator of known Lp(a) concentration

ESTIMATION OF M.D.A[12]

Lipoproteins were precipitated from the specimen by adding 20% TCA. Then specimen was treated with TBA (Thiobarbituric Acid) in sodium sulphate to form chromogen. This chromogen is allowed to form a complex in boiling water bath and extracted in butanol which is measured at 530 nm.

ESTIMATION OF BLOOD GULTATHIONE (BEUTLER 1963)[13]

Glutathione (GSH) in the whole blood or red blood cells is maintained in reduced state through reduced Nicotinamide adenine dinucleotide phosphate and Glutathione reductase. The functions of reduced glutathione seen to be to keep sulfhydryl groups in their active reduced state and through Glutathione peroxidase to remove hydrogen peroxide. Photometric method adapted by Beutler (1963) using 5-5' Di-thiobis 2-Bitro benzoic acid (D.T.N.B.) was used for the assay of Blood Glutathione levels. The method is based upon the development of a relatively stable yellow color when D.T.N.B. is added to sulfhydryl compound.

VITAMIN E

DETERMINATION OF SERUM TOCOPHEROL (BAKER AND FRANK, 1968)[14]

Serum tocopherols can be measured by their reduction of ferric to ferrous ions which then form a red complex with α, α' – dipyridyl. Tocopherols and carotenes were first extracted into xylene and the absorbance is read at 460 nm to measure the carotenes. A correction for the carotenes is made after adding ferric chloride and reading at 520 nm.

VITAMIN C

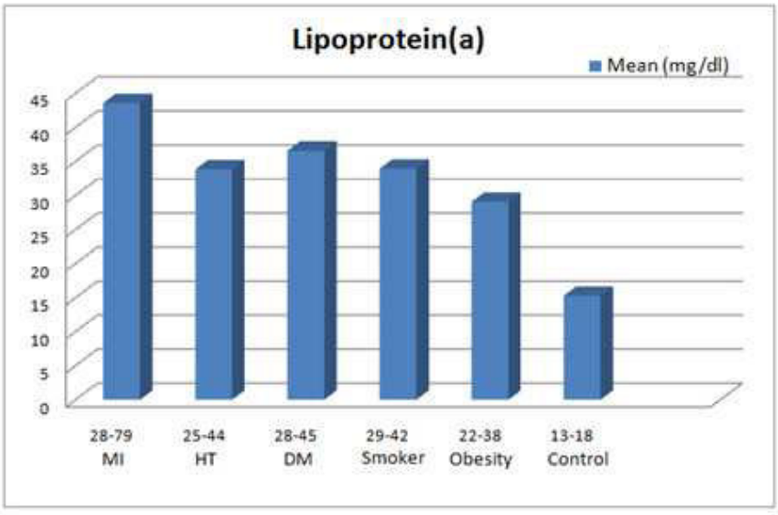
DETERMINATION OF PLASMA ASCORBATE BY 2,6-DICHLOROPHENOLINDOPHENOL TITRATION[15]

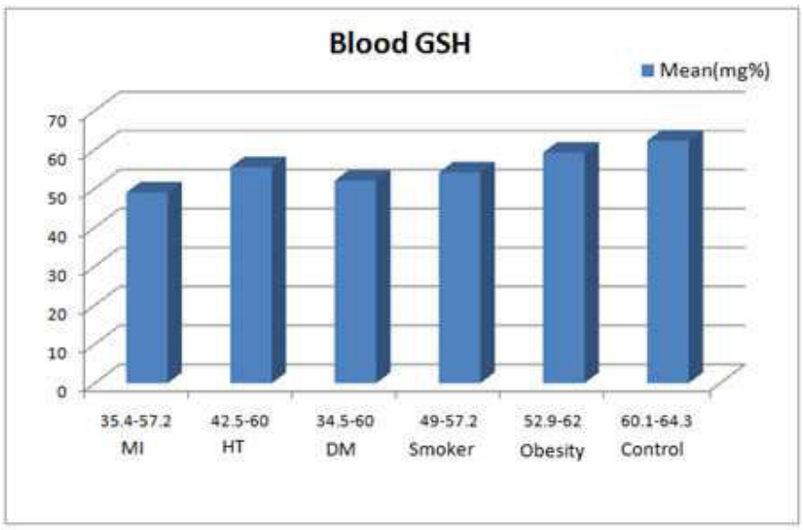
DETERMINATION OF RETINOL AND CAROTENES IN SERUM USING THE CARR-PRICE REACTION[16]

Proteins are precipitated with ethanol and the retinol and carotenes extracted into light petroleum. After reading the intensity of yellow colour due to the carotenes the light petroleum is evaporated off and the residue dissolved in chloroform. Carr-Price reagent is added and the amount of blue colour produced read. Since carotenes also give some colour, a correction for this is made in order to obtain that due to the Retinol present. The present study comprises of 100 subjects, which includes 80 cases of Coronary heart diseases of which 20 cases of Myocardial Infarction (MI), 60 cases with risk factors for CHD including Hypertension (HT), Diabetes mellitus (MI), Smoking and Obesity (Table 1) and 20 age matched controls.

Group	Number of cases
Myocardial Infarction	20
Hypertension	15
Diabetes Mellitus	15
Smokers	15
Obesity	15
Control	20
Total	**100**

Table 1
Distribution of Total Study Subjects





Group	Lp(a) (mg/dl)	MDA (µmol/l)	GSH (mg%)	VIT E (mg/l)	VIT C (mg/l)	VIT A (mg/l)
Myocardial Infarction (20)	43.59±10.22	15.98±2.06	48.94±5.57	5.33 ± 1.12	5.75 ± 0.91	0.31 ± 0.04
Hypertension (15)	33.83±6.29	12.64±2.64	55.46±4.65	5.98 ± 1.33	6.76 ± 1.05	0.33 ± 0.04
Diabetes Mellitus (15)	36.57±5.29	13.77±2.17	52±6.61	6.28 ± 1.88	6.72 ± 1.69	0.33 ± 0.03
Smokers (15)	33.93±3.57	13.3±1.12	54.08±2.16	4.90 ± 0.32	6.02 ± 0.54	0.37 ± 0.04
Obesity (15)	29.07±3.42	11.68±1.09	59.17±2.21	5.88 ± 0.48	7.48 ± 0.65	0.48 ± 0.08
Control (20) (Reference group)	15.27±1.31	6.76±1.20	62.28±1.42	12.74 ± 1.34	11.26 ± 1.37	0.47 ± 0.08
MI	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
HT	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
DM	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Smokers	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Obesity	*P<0.001*	*P<0.001*	P>0.05	*P<0.001*	*P<0.001*	P>0.05

Table 2

Table Showing the Blood level of Special Parameters in cases and controls.

Group	Cholesterol (mg%)	TG (mg%)	HDL (mg%)	LDL (mg%)
Myocardial Infarction (20)	234.0 ± 29.12	180.4 ± 24.89	35.75 ± 3.25	193.5 ± 14.56
Hypertension (15)	234.4 ± 25.30	153 ± 23.28	40.07 ± 3.55	165.8 ± 19.71
Diabetes Mellitus (15)	226.9 ± 38.03	177.6 ± 29.88	40.6 ± 3.92	171.7 ± 8.21
Smokers (15)	229.4 ± 34.11	166.6 ± 18.85	35.89 ± 4.59	160.7 ± 18.68
Obesity (15)	26.01 ± 29.42	198.0 ± 21.61	40.2 ± 3.61	207.2 ± 14.09
Control (20) (Reference group)	178.2 ± 7.81	133.1 ± 5.65	55.8 ± 5.55	128.9 ± 6.36
MI	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
HT	*P<0.001*	P>0.05	*P<0.001*	*P<0.001*
DM	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Smokers	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Obesity	*P<0.001*	*P<0.001*	P>0.05	*P<0.001*

Table 3

Table Showing the Blood level of Routine Parameters in cases and controls.

Group	VLDL. (mg%)	TC/LDL ratio	LDL/HDL ratio	Bl. Glucose
Myocardial Infarction (20)	39.8 ± 5.21	6.51 ± 0.68	5.38 ± 0.38	104.1 ± 10.76
Hypertension (15)	32.7 ± 7.81	5.84 ± 0.70	4.10 ± 0.48	105.6 ± 9.98
Diabetes Mellitus (15)	35.0 ± 10.36	5.50 ± 0.95	4.24 ± 0.39	204.2 ± 42.37
Smokers (15)	41.6 ± 4.33	6.27 ± 0.92	4.60 ± 0.74	108.2 ± 10.00
Obesity (15)	53.6 ± 11.74	6.45 ± 0.83	5.14 ± 0.51	110.2 ± 6.10
Control (20) (Reference group)	28.6 ± 2.43	3.17 ± 0.37	2.28 ± 0.19	104.1 ± 10.76
MI	*P<0.001*	*P<0.001*	*P<0.001*	*P>0.05*
HT	*P>0.05*	*P<0.001*	*P<0.001*	*P>0.05*
DM	*P>0.05*	*P<0.001*	*P<0.001*	*P<0.001*
Smokers	*P<0.001*	*P<0.001*	*P<0.001*	*P>0.05*
Obesity	*P<0.001*	*P<0.001*	*P<0.001*	*P>0.05*

Table 4

Table Showing the Blood level of Routine Parameters in cases and controls.

RESULTS AND DISCUSSION

The analysis of study group revealed that the Coronary heart diseases cases were between 30 to 60 year. In Myocardial infarction cases, 51 to 60 years group maximum with 55% followed by 41 to 50 years group with 35%. Age matched controls, 51 to 60 years group maximum with 45% followed by 41 to 50 years group with 30%. It has been long known that ageing has a steady and consistent with atherosclerotic lesion. It is universally accepted that men are more prone to coronary atherosclerosis than women of child bearing age .After menopause, however women have equal risk of developing CHD. Majority of the cases were from upper class with 50% followed by middle class with 30% . As observed by many workers, the incidence of CHD is higher in person with high Socio-Economic status, while it is lower in low socio-economic groups. Majority of patient of myocardial infarction, 90% presented with chest pain followed by, 80% with sweating, 45% with vomiting, 30% with breathlessness, 40% with palpitation and 20% with giddiness . Results of routine investigation shows an increase in serum cholesterol, serum LDL, TC/HDL and LDL/HDL ratio were seen in all five groups including MI, HT, DM, Smokers and Obesity cases, which were statistically significant. Serum Triglycerides were increased in MI, DM, Smokers and Obesity cases, which were statistically significant, where as significant increase was not seen in HT cases. Serum VLDL showed a statistically significant increase in MI, Smokers and Obesity cases, where as significant increase was not seen in HT and DM cases. Blood glucose showed a significant increase in DM cases, where as no significant increase were seen in MI, HT, Smokers and Obesity cases. A statistically significant decrease in serum HDL were seen in all five groups including MI, HT, DM, Smokers and Obesity cases.

In special investigations, Lipoprotein (a) levels are increased in all five group including MI, HT, DM, Smokers and Obesity in comparison with levels in normal healthy controls. The similar results were obtained by Boston[17], Sarah[18], Assman[19], Leo[20], Matthias[21], Rajeshekhar[22], who demonstrated a rise in serum Lipoprotein(a) levels in cases of CHD as compared to controls and who reported that elevated Lp(a) is an independent risk factor for the development of CHD. Serum MDA levels were found to be higher in cases as compared to controls. This could be due to increased oxidative stress. We have also

found a positive correlation between severity of disease and MDA levels. Simmi[23] reported a significant increase in MDA level in acute myocardial infarction. Giardina [24] studied MDA level as a marker of lipid peroxidation. Vijay[25] studied a relationship between lipid peroxidation and CHD Vitamin E and Vitamin C levels were decreased in all five groups including MI, HT, DM, Smokers and Obesity in comparison with levels in normal healthy controls Glutathione and Vitamin A levels were decreased in four groups including MI, HT, DM and Smokers in comparison with levels in normal healthy controls, where as in Obesity group the levels were not significant . Blood Glutathione, Serum Vitamin E, plasma Vitamin C and plasma Vitamin A levels were studied in patients of CHD and in patients with risk factor for CHD, as a measure of antioxidant status. Results showed a highly significant decrease in Glutathione, α- tocopherol, ascorbic acid and Vitamin A levels in cases as compared to controls. This could be indicative of increased need and a defective antioxidant mechanism in order to overcome the oxidative stress. An increased tendency to peroxidation of polyunsaturated fatty acids resulting from a reduction in anti oxidant availability might favour thrombosis.

John [26] reported GSH as an protective factor against the development of atherosclerosis and low GSH is an significant predictor of parental CHD. Calvin [27] reported a marked decrease in GSH in the cardiovascular diseases. Marx [27] studied LDL deposited under endothelial cells of arterial wall might undergo oxidation by oxygen free radicals. Esterbauer[28] said that atherosclerosis believed to be oxidative modification of LDL. Debra [29] described that antioxidants may protect against lipoprotein oxidation and inhibit atherosclerosis and its clinical sequelae. Pau [30] and Prabhat Jha [31] reported intake of antioxidant vitamins such as carotene, vitamin C and vitamin E preventing against CHD. Jialal [32] studied vit C, E and A inhibit LDL oxidation. In Hypertension group, serum MDA and serum Lipoprotein (a) levels are elevated and Blood Glutathione, serum Vitamin E, plasma Vitamin C and plasma Vitamin A levels were diminished, as compared to controls. In smokers group, serum MDA and Serum Lipoprotein (a) values were increased and Blood Glutathione, serum Vitamin E, plasma Vitamin C and plasma Vitamin A values were diminished, as compared to controls. In obesity group, serum MDA and Serum Lipoprotein (a) levels were elevated and serum Vitamin E and plasma Vitamin C levels were diminished, where as Blood Glutathione and plasma Vitamin A levels were not significant as compared to controls. Thus, there were alterations in MDA, Lipoprotein (a) and anti-oxidant levels in cases of Coronary heart diseases and also in cases with risk factor for Coronary heart disease including hypertension, diabetes mellitus, smokers and obesity .

CONCLUSION

From the results, it can be concluded that serum MDA can be used to detect severity of coronary heart diseases, as we have observed a significant increase in blood MDA and a significant decrease in blood glutathione, Vitamin E, Vitamin C and Vitamin A with the increase in severity of oxidative stress. A highly statistically significant increase in the serum Lipoprotein (a) was seen in cases as compared to controls. So we can conclude that Lipoprotein (a) is an independent risk factor for Coronary heart diseases. It is therefore, concluded that MDA, Lipoprotein (a) and anti-oxidants like Glutathione, Vitamin E, Vitamin C and Vitamin A, be evaluated as bio-chemical parameters for preclinical assessment of “at risk group” for Coronary heart diseases and for assessing and monitoring cases of Coronary heart diseases. Further studies with anti-oxidant Vitamin supplementation have to be performed to test the nature of association between high MDA, high Lipoprotein (a) and low anti-oxidants in Coronary heart diseases. And also further studies are required to evaluate the significance of serum Lipoprotein (a) estimation in the assessment of CHD risk of human subjects.

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