ROLE OF BILIRUBIN, VITAMIN C AND CERULOPLASMIN AS ANTIOXIDANTS IN CORONARY ARTERY DISEASE [CAD].

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ABSTRACT

A study has been carried out on 250 CAD patients to see how the serum levels of three antioxidants i.e., Vitamin C, Bilirubin and Ceruloplasmin are related to the CAD risk factors and characteristics in these patients. The number of severe category CAD patients declined by 7-18% with increasing serum levels of the three antioxidants and, triple vessel disease declined by 14-20%. A decline of 39% in Myocardial Infarction (MI) occurred with increasing serum Ceruloplasmin. Serum Ceruloplasmin was significantly lower in the MI group compared to the non MI group CAD patients. There was a steady and a significant decline in the mean values of serum levels of cholesterol, Triglyceride, VLDL cholesterol along with BMI with increasing serum level. The study indicates that with increasing serum levels of the three antioxidants studied, the % MI along with % Triple vessel disease and severity of CAD goes down suggesting that the modifiable risk factors have to be suitably modified in order to maintain a reasonably high level of these antioxidants, as the risk factors are inversely related to the serum antioxidant levels.

KEY WORDS

Antioxidants, Bilirubin, Ceruloplasmin, CAD, Role, Serum Levels, Vitamin C.

INTRODUCTION

Coronary artery disease [CAD] remains the major cause of morbidity and mortality in all developed countries in the world and in India, it has already climbed the 'Charts' from 14th to 4th place only behind tuberculosis, communicable diseases and malnutrition. Lipids and lipoproteins are important risk factors for CAD; however, they do not account for the disease in 30 to 40% of the population with CAD (1). Other risk factors include smoking, hypertension, diabetes mellitus etc. (2). Major CAD risk factors do not predict subsequent myocardial infarction accurately and do not fully explain social class differences in Indians as Indians have a high incidence of CAD which is not fully explained by conventional risk factors. The search for CAD risk factors that might explain these variations has been stimulated by the evidence that free radicals are involved in the pathogenesis of atherosclerosis (3) and that antioxidants, both dietary and endogenous, may be important protective factors (4).

Schwertner *et al.* in 1994 showed that serum bilirubin is an inverse and independent risk factor for CAD (5). They examined serum bilirubin along with various liver enzymes as possible risk factors for angiographically confirmed CAD in 619 men with complete data for the risk factors considered and in 258 men with incomplete data for the various risk factors. In their study, all subjects were U.S. Air Force pilots and from statistical analysis of the data according to the various hypotheses, the authors deduced that a 50% decrease in the bilirubin was associated with a 47% increase in the possibility of being in a more severe CAD category. Later, Breimer *et al.* in 1995 (6) observed a U-shaped relationship between serum bilirubin and the risk of IHD in a prospective study of 7685 middle aged British men.

The antioxidant property of ceruloplasmin is through its oxidase activity, which is directed towards aromatic amines, phenols (7) as well as towards ferrous ions (ferroxidase activity) (8). It also inhibits ferrous ion stimulated lipid peroxidation and is known to be involved in the decomposition of lipid peroxides (9).

Enstrom *et al.* in 1992 (10) in a prospective study involving 11,348 adults showed an inverse relationship between Vitamin 'C' intake and overall cardiovascular morbidity. Nyyssonen *et al.* in 1997 (11) on a prospective population study of 1605 healthy men aged 42, 48, 54 or 60 year in Finland showed that men

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who had Vitamin 'C' deficiency had a relative risk of myocardial infarction, 2.5 fold greater compared to men with higher plasma vitamin C concentration after adjustment for other risk factors.

MATERIAL AND METHODS

□ The study group consisted of 250 CAD patients who were selected after proper clinical evaluation and investigations. All the patients with liver disease, renal disease, gout, thyroid disease and Wilson's disease were excluded from the study, as well as any patient who was on antioxidant supplementation or lipid lowering therapy.

- 'P' values were calculated by unpaired 't' test.
- CAD severity was divided into three categories, based on % stenosis.

Category	% stenosis
Severe	> 70%
Moderate	50-70%
Mild	< 50%

Assays were done as under :

- Total and direct bilirubin (Diazotised sulfanilic acid method) (12).
- Vitamin C (Dichlorophenol indophenol decolorisation method) (13).
- Ceruloplasmin (Para phenylene diamine method, Ravin, 1961) (14).

RESULTS

Table 1 lists the distribution pattern of nine major CAD risk factors (both modifiable and non-modifiable) in the 250 CAD patients in this study.

Bilirubin

There was a significant decrease in the serum levels of total cholesterol, triglyceride and VLDL cholesterol, with increasing serum bilirubin (Table 2).

Also a decline of 7.5% in the moderate CAD category patients occurred with increasing levels of serum bilirubin, along with a decrease of 14% in TVD and 8% in SVD (Table 3).

Vitamin C

A steady and a significant decline occurred in the BMI values of CAD patients with increasing levels of serum vitamin C (Table 4).

Also, a 15% decline occurred in the severe category

CAD patients with increasing serum vitamin C levels, along with a 20% decline in TVD (Table 5).

Ceruloplasmin

There was a steady and a significant decline in the BMI and serum total cholesterol values, with increasing serum levels of ceruloplasmin in CAD patients (Table 6).

Also, a decline of 18% in the number of severe CAD category patients occurred with increasing levels of serum ceruloplasmin along with 15% in TVD (Table 7).

A steady decline of 39% in % MI occurred with increasing levels of serum ceruloplasmin (Table 8).

Serum levels of ceruloplasmin, was significantly lower in the MI group compared to the non-MI group CAD patients. No significant difference was observed between the two groups, in terms of serum levels of vitamin C, and total bilirubin. (Table 9).

DISCUSSION

Vitamin C

The steady and a significant decline of BMI with increasing serum vitamin C levels indicates a BMI dependent consumption of vitamin C for endogenous antioxidant purposes in CAD patients.

Enstrom *et al.* in 1992 (10) have shown an inverse relationship between vitamin C intake and overall cardiovascular mortality in a population study involving 11,348 adults.

In another study, Jialal *et al.* in 1990 (15) have shown that at physiological level, vitamin C and alpha-tocopherol both could inhibit the oxidative modification of LDL following a five hour incubation. Only vitamin C continued to inhibit the oxidative modification to a significant degree even at 24 hours and concluded that these findings could have major implications with respect to the *in vivo* prevention of oxidative modification of LDL by vitamin C. In the present study also, a 15% decline in the severe CAD category patients as well as a 10% decline in the CAD positive family history was observed with increasing levels of serum vitamin C. With the total % of severe, moderate, mild as well as TVD, DVD, SVD fixed at 100%, any decrease in severe CAD category and % TVD, with increasing levels of the antioxidant, vitamin C, would be accompanied by an increase in the other two groups. This indicates that vitamin C has a protective role to play in severe CAD category patients and Triple vessel disease (TVD) groups.

Although Nyyssonen *et al.* in 1997 (11) have shown in a prospective population study of 1605 healthy men aged 42,48,54 or 60 years in Finland that men who had vitamin C deficiency had a relative risk of MI 2.5

Table 1. Risk factor distribution pattern of the CAD patients in this study

S. No.	Risk factor	n=250	
1.	Serum Total	100-150	116 (46%)
	Cholesterol (mg/dl)	141-396	134 (54%)
2.	Serum triglyceride	59-150	125 (50%)
	(mg/dl)	151-463	125 (50%)
3.	BMI (kg/m ²)	21-25	131 (52%)
		25.1-30	119 (48%)
4.	Smokers		107 (43%)
	Non-smokers		143 (57%)
5.	Diabetics		80 (32%)
	Non-diabetics		170 (68%)
6.	Hypertensives		122 (49%)
	Non-hypertensives		128 (51%)
7.	Age (years)	60-89	83 (33%)
		50-59	108 (43%)
		31-49	69 (24%)
8.	Sex	Male	218 (87%)
		Female	32 (13%)
9.	CAD Family History	Positive	46 (18%)
		Negative	204 (82%)

Table 2. Relationship of serum bilirubin levels with modifiable CAD risk factors

Risk factor	Serum Bilirubin level (mg/dl)	
	0.10-0.60 n=130	0.61-1.30 n=120
Serum total cholesterol, mg/dl (Mean)	161.66	152.39 (p<0.025)
Serum triglycerides, mg/dl (Mean)	163.6	152.46 (p<0.025)
VLDL cholesterol, mg/dl (Mean)	32.72	30.49 (p<0.025)

Table 3. Serum bilirubin - CAD severity relationship

Risk factor	Serum Bilirubin level (mg/dl)	
	0.10-0.60 n=106	0.61-1.30 n=92
Severe (>70)	88 (83%)	83 (90%)
Moderate (50-70%)	10 (9.5%)	02 (02%)
Mild (<50%)	08 (7.5%)	07 (08%)
Triple vessel disease (TVD)	38 (36%)	21 (22%)
Double vessel disease (DVD)	21 (30%)	47 (52%)
Single vessel disease (SVD)	36 (34%)	24 (26%)

Table 4. Relationship of serum vitamin C levels with modifiable CAD risk factors

Risk factor	Serum vitamin C level (mg/dl)			
	0.10-0.30 n=57	0.31-0.60 n=63	0.61-0.90 n=75	0.91-1.8 n=55
Serum total cholesterol, mg/dl (Mean)	158.19	153.17	160.62	158.68
Serum triglycerides mg/dl (Mean)	150.35	158.87	178.41	172.43
VLDL cholesterol, mg/dl (Mean)	26.12	25.25 NS*	24.40 p<0.05	23.65 p<0.025
* Not significant.				

Table 5. Vitamin C-CAD severity relationship

CAD severity*	Serum vitamin C level (mg/dl)			
	0.10-0.30 n=57	0.31-0.60 n=63	0.61-0.90 n=75	0.91-1.8 n=55
Severe (>70)	49 (96%)	41 (87%)	46 (81%)	35 (81%)
Moderate (50-70%)	01 (02%)	03 (6.5%)	05 (09%)	03 (07%)
Mild (<50%)	0.1 (02%)	03 (6.5%)	06 (10%)	05 (12%)
Triple vessel disease (TVD)	20 (39%)	18 (38%)	13 (23%)	08 (19%)
Double vessel disease (DVD)	17 (33%)	18 (38%)	26 (46%)	18 (42%)
Single vessel disease (SVD)	14 (28%)	11 (24%)	18 (31%)	17 (39%)
* Angiography findings were available only in 198 patients				

compared with men with higher plasma vitamin C concentration, no direct relationship was observed in our study between % MI and serum vitamin C level, but BMI, and severity of CAD showed an inverse relationship with serum vitamin C.

Ceruloplasmin

Mateeseu *et al.* in 1995 (1) have shown in their studies that ceruloplasmin could protect the myocardial tissue against the deleterious effects of oxygen free radical (OFR). Ceruloplasmin, which is a chain breaking antioxidant, had an inverse relationship with % MI as there was steady decline of 39% in %MI with it's increasing levels in serum in our study. Also, there was

a steady and a significant decline in BMI and serum cholesterol value along with a decreasing trend in CAD severity (18%) and TVD (19%) with increasing levels of this antioxidant. The decreasing trend in severe CAD category of TVD CAD patients with increasing levels indicates the protective role played by Ceruloplasmin in these two categories.

Atanasiu *et al.* in 1995 (17) were also able to demonstrate the anti-arrhythmic effects of ceruloplasmin in the ischemic rat heart as it could reduce the incidence of ventricular fibrillation at reperfusion. The inverse relationship between serum ceruloplasmin and the five CAD risk factors / characteristics mentioned above, indicate the

Table 6. Relationship of serum ceruloplasmin levels with modifiable CAD risk factors

Risk factor	Serum ceruloplasmin level (U/L)		
	80-1000 n=67	1001-2000 n=121	2001-6000 n=62
Serum total cholesterol, mg/dl (Mean)	166.46±5.12	152.43±4.80 p<0.05	149.70±3.92 p<0.05
Serum triglycerides mg/dl (Mean)	170.40±18.16	156.52±20.12	167.12±17.18
VLDL cholesterol, mg/dl (Mean)	25.91±1.18	25.00±1.12 NS*	23.83±1.05 p<0.05
* Not significant.			

Table 7. Serum ceruloplasmin-CAD severity relationship

CAD severity*	Serum ceruloplasmin level (U/L)		
	80-1000 n=67	1001-2000 n=121	2001-6000 n=62
Severe (>70)	60 (92%)	72 (90%)	39 (74%)
Moderate (50-70%)	03 (05%)	04 (05%)	05 (10%)
Mild (<50%)	02 (03%)	04 (05%)	09 (16%)
Triple vessel disease (TVD)	25 (38%)	24 (30%)	10 (19%)
Double vessel disease (DVD)	28 (42%)	28 (35%)	24 (45%)
Single vessel disease (SVD)	13 (20%)	28 (35%)	19 (36%)

cardioprotective nature of this antioxidant, as shown by previous workers.

Bilirubin

Schwertner *et al.* (5) have shown in their study on 877 men that serum bilirubin had an inverse relationship with age, serum cholesterol, cigarettes smoked/day, systolic BP, serum triglyceride and fasting glucose. Later, Breimer *et al.* (6) have also shown in a prospective study of 7685 middle-aged men that serum bilirubin had an inverse relationship with age, % smokers, % antihypertensive drugs, and serum cholesterol. In our study also, serum cholesterol, serum triglyceride and serum VLDL cholesterol showed an inverse relationship with serum bilirubin.

Also, the decreasing trend in moderate CAD category, TVD and SVD by 7.5%, 14% and 12% respectively

with increasing serum levels of this antioxidant indicates the protective role played by the same in the pathogenesis of CAD. This indicates that bilirubin as an antioxidant plays a protective role in moderate CAD category patients.

Oxidative stress, arising as a result of an imbalance, between free radical production and antioxidant deficiencies, is associated with damage to a wide range of molecular species, which includes lipids and lipoproteins also. Atherosclerosis is a process for which there is substantial evidence of a role for oxidative stress. Hypercholesterolemia is universally accepted as a major risk factor for atherosclerosis, but at any given concentration of plasma cholesterol, there is variability in the occurrence of cardiovascular events, as it has been shown that the oxidative modification of LDL might be a crucially important step

Table 8. Serum ceruloplasmin - % MI relationship

	Serum ceruloplasmin level (U/L)		
	80 1000 n=67	1001- 2000 n=121	2001- 6000 n=62
MI	49 (73%)	58 (49%)	21 (34%)
Non MI	18 (27%)	53 (51%)	41 (66%)

in the development of atherosclerotic plaque (18).

Evidence that endogenous and exogenous antioxidants potentially reduce the risk of CAD comes from the following :

1. Studies of antioxidant supplementation in animal models of atherosclerosis have shown a reduction in the disease process (19).
2. Many studies have shown that antioxidant supplementation in healthy subjects or CAD patients can reduce levels of free radical damage produced and protect LDL against oxidation (20).
3. Large scale epidemiological studies have shown that low intake of antioxidants are associated with increased cardiovascular risk (21).

Schwertner *et al.* (5) suggested that the relation of increased CAD risk with low bilirubin was a reflection of its 'consumption' in endogenous antioxidant activity. The roles and relative importance of endogenous antioxidants like bilirubin, ceruloplasmin and exogenous ones like Vitamin C, are not well understood. It is possible that an interaction takes place between endogenous and exogenous systems that in people with a deficient dietary intake of exogenous antioxidants, a compensatory increase in endogenous antioxidant production occurs. Thus, it is possible that in individuals with a low intake of exogenous antioxidants, these endogenous antioxidants might be compensating for the deficiencies and act as markers of dietary antioxidant deficiency. The antioxidants may be operating in complex ways modulated by each other. Thus, these exogenous and endogenous antioxidants might be operating both in the early phase of development of atherosclerosis as well as in the period closer to the onset of clinical events.

Table 9. Serum antioxidant levels in Non-MI and MI group CAD patients

Risk factor	Serum level (Mean±SD)	
	Non MI Group n=122	MI Group n=128
Vitamin C (mg/dl)	0.675±0.061	0.660±0.056
Ceruloplasmin (U/L)	1875.72±110.10	1426.98±91.20 p<0.01
Total bilirubin (mg/dl)	0.626±0.051	0.653±0.05

CONCLUSION

The study indicates that with increasing serum levels of the three antioxidants studied, the % MI along with % Triple vessel disease and severity of CAD goes down suggesting that the modifiable risk factors have to be suitably modified in order to maintain a reasonably high level of these antioxidants, as the risk factors are inversely related to the serum antioxidant levels.

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