OXIDANT STATUS AND LIPID PROFILE IN VEGETARIANS AND FISH EATERS

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ABSTRACT

Habitual consumption of moderate amounts of fish is associated with reduced mortality from coronary heart disease. However the beneficial effects of fish enriched diet seem contradictory, due to the susceptibility of the PUFAs in them to oxidation. It is also acclaimed that vegetarians in general, have a lower serum total cholesterol and LDL-cholesterol and a better antioxidant status. This contradiction was the basis for the study, where the validity of the above finding was tested in a general population consuming a vegetarian or predominantly fish diet as a regular dietary habit. The oxidant status and the lipid profile of 23 vegetarians and 22 fish eaters was studied by estimating the plasma lipid peroxides measured as malondialdehyde (MDA) and the lipid parameters viz. Total Cholesterol (TC), HDL-Cholesterol (HDL-C) and triglycerides (TG). Statistical analysis was carried out to compare the MDA values and the lipemic status between the two groups. MDA was also correlated with the various lipid indices among the study groups. None of the compared and correlated values was statistically significant, although certain altered trends were seen. From this study, it may be concluded that oxidant status and lipid profile does not vary significantly in the two groups, suggesting that neither a vegetarian diet nor a fish diet has a definite benefit over the other.

KEY WORDS

Free radicals, High Density Lipoprotein – Cholesterol, Malondialdehyde, Triglycerides

INTRODUCTION

Dietary fat plays an important role in heart diseases by affecting atherogenesis. Epidemiological studies on Greenland Eskimos have shown a correlation between low incidence of coronary heart disease (CHD) and high consumption of fish products (1). Subsequent studies have also confirmed these findings (2,3). It is well established that free radical damage contributes to the etiology of cardiovascular disease as well as other diseases such as diabetes mellitus, cataract etc. There is growing interest in the possible role of free radicals in the development of CHD. Free radicals can cause oxidation of low density lipoproteins (LDL). Oxidised LDL has been demonstrated to accelerate endothelial damage, macrophage recruitment and increased uptake of low density lipoprotein-cholesterol (LDL-C) by foam cells which gradually develops into fatty streaks. Coronary artery disease is more common among males, affecting five times more males than females under

45 years of age. It has been established beyond reasonable doubt that lowering the elevated blood cholesterol level will reduce the risk of CHD.

Habitual consumption of moderate amount of fish may be associated with reduced mortality from CHD and is attributed to the n-3 fatty acids present in them (3). Bang and Dyerberg emphasized the importance of n-3 fatty acids in the prevention of myocardial infarction because of its antithrombotic and hypocholesterolemic effect (4,5).

On the other hand, vegetarians have a greater intake of n-6 series of fatty acids in their diet. Differences in blood lipids between the vegetarians and omnivores have been well studied (6,7). It is shown that vegetarians in general have a lower serum total cholesterol (TC) and LDL-C than do omnivores (8). This has been explained by a high dietary polyunsaturated : saturated fatty acid ratio (P:S), low dietary fat and cholesterol intake and high fibre intake in vegetarians. Also there is an additional benefit of micronutrients and antioxidants in the vegetarian food (9). The work done by the same authors on the antioxidant and oxidant status of vegetarians and fish

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eters has shown significant increase in ascorbic acid levels in vegetarians (10).

Enhanced susceptibility of the membranes to peroxidation due to the increased PUFA content seems to contradict the beneficial effects of fish enriched diet. Oxidation of PUFA and depleted content of antioxidants may increase the formation of peroxides and toxic aldehydes (11,12). It has also been shown that hypercholesterolemia produces superoxide anion (13). Such undesirable effects may be caused by the consumption of unsaturated vegetable oils also. However, with greater quantity of natural antioxidants present in vegetable oils, these effects are less pronounced. Information on the oxidant status and lipid parameters in fish eaters and vegetarians is meagre. The 40 to 55 years age group is a most susceptible age for myocardial infarction, particularly in males as the females are protected by estrogen. Thus an attempt has been made to

(i) compare the blood lipids and the oxidant status in fish eaters and vegetarians.

(ii) evaluate the correlation between plasma MDA and lipid status in vegetarians and fish eaters.

MATERIALS AND METHODS

Twenty three vegetarians and twenty two predominantly fish eating men in the age group of 40-55 years were chosen for the study. The inclusion and exclusion criteria were as follows.

Inclusion criteria:

Vegetarians: Subjects consuming purely lacto vegetarian diet.

Fish Eaters: Subjects consuming 4-6 fish dishes per week (4-6 fish meals/ week) along with vegetables and occasionally other meats (An approximate quantity was calculated through our questionnaire, by dividing the quantity of fish cooked by the number of family members consuming it. The average amount was almost the same in all the volunteers).

Exclusion criteria:

For both groups, subjects who were smokers, alcoholics, diabetics or hypertensives were excluded from the study.

An informed consent was taken from the volunteers. Age, height, weight and blood pressure were recorded and food habits of the subjects were obtained using a questionnaire.

5 ml of heparinised venous blood was collected after an overnight fast of 12 hrs and the following parameters were estimated in the plasma within 2 hrs of sample collection.

1) Malondialdehyde (MDA)

2) Total cholesterol (TC)

3) High density lipoprotein-cholesterol (HDL-C)

4) Triglycerides (TG)

Plasma lipid peroxide was estimated as given in our earlier communication (10) as thiobarbituric acid (TBA) reactive substance, malondialdehyde, by Yagi's method (14) with modifications (15).

Plasma total cholesterol was estimated using 'Human Diagnostics' manual kit by CHOD-PAP method which involves the enzymatic procedure of Allain *et al* (16). The intensity of the red colour developed was measured at 500 nm.

For the estimation of HDL-C, the procedure of Burnstein *et al* (17) was followed. The principle of the method involves the precipitation of chylomicrons, LDL-C and VLDL-C by phosphotungstic acid - $MgCl_2$ precipitating reagent. Centrifugation leaves only the HDL-C in the supernatant. The cholesterol content in the HDL fraction is estimated as done for TC.

Estimation of plasma triglycerides was done using 'Human Diagnostics Kit' by the GPO - PAP method (18,19). The enzymatic procedure of Werner *et al* was followed. The intensity of the pink colour developed was measured at 500 nm. LDL-Cholesterol was calculated using Friedwald's and Fredrickson's formula (20) which assumes that VLDL-C is present in a concentration equal to 1/5th the triglyceride concentration.

$$LDL-C = TC - \left(\frac{TG}{5} + HDL-C \right)$$

Non HDL cholesterol (21) is defined as the difference between TC and HDL-C and includes all the cholesterol present in lipoprotein particles considered to be atherogenic. It was calculated as [TC-HDL-C]. Risk ratios were calculated as TC/HDL-C, LDL-C/HDL-C and Non HDL-C/HDL-C.

STATISTICAL ANALYSIS:

The students 't' test or Mann-Whitney 'U' test was applied depending upon the nature of the data. Pearson's correlation coefficients were used to compare the correlations.

RESULTS:

Anthropometric data of the two groups are presented in Table 1. Both the groups were comparable in age

and BMI. All the subjects were normotensive. The oxidative stress assessed by estimating the MDA and the values of lipid parameters are presented in Table 2.

The mean content of plasma MDA is $4.78 \pm 2.65 \mu\text{mol/L}$ in vegetarians and $4.11 \pm 1.93 \mu\text{mol/L}$ in fish eaters. The difference between the two groups is not statistically significant. Results of the lipid parameters viz Triglycerides (TG), total cholesterol (TC), HDL – cholesterol (HDL-C), LDL – cholesterol LDL – C and

The correlation of MDA with HDL-C is negative in the vegetarians ($r = -0.242$) and weakly positive in the fish eaters ($r = +0.022$). Although LDL-C and Non HDL-C showed a positive correlation with MDA in both the groups, the association was stronger in vegetarians. Triglycerides also showed a positive correlation with MDA and correlation was stronger in fish eaters ($r = 0.207$ Vs 0.025).

The risk ratios calculated as TC/HDL-C, LDL-C/HDL-

Table 1. Anthropometric data

	Vegetarians (n=23)	Fish Eaters (n=22)
Age (years)	45.95 ± 4.33	46.05 ± 4.14
Body Mass Index	27.17 ± 3.74	27.97 ± 3.32
Systolic blood pressure (mmHg)	125.40 ± 10.08	127.18 ± 9.15
Diastolic blood pressure (mmHg)	82.78 ± 6.29	85.23 ± 5.11
n = number of subjects values are Mean $\pm$ SD		

Table 2. Plasma lipid peroxides and Lipid profile of vegetarians and fish eaters

	Vegetarians (n=23)	Fish Eaters (n=22)
MDA ($\mu\text{mol/L}$)	4.78 ± 2.64	4.10 ± 1.93
TC (mg/dl)	187.50 ± 48.77	202.04 ± 44.03
HDL – C (mg/dl)	46.49 ± 11.87	50.15 ± 18.36
LDL – C (mg/dl)	101.67 ± 57.47	113.34 ± 57.28
Non HDL-C (mg/dl)	139.98 ± 51.53	151.91 ± 45.26
Triglycerides (mg/dl)	196.84 ± 121.70	190.53 ± 139.32
n = number of subjects values are Mean $\pm$ SD		

Non HDL – cholesterol (non HDL-C) also do not show any significant difference between the vegetarians and fish eaters. All the values in both the groups were well within the normal limits, with the exception of TG and MDA levels. (TC < 200mg/dL, LDL – C < 130mg/dL, HDL – C > 40mg/dL, Non HDL – C < 160mg/dL). The TG values were > 160mg/dL (60-160mg/dL) and that of MDA were > 3.8 $\mu\text{mol/L}$ (1.9 – 3.8mmol/L).

The 'r' values for the correlation of MDA with lipid parameters are given in Table 3. A positive correlation of MDA with total cholesterol is seen in both the groups.

Table 3. Correlation of MDA with lipid parameters ('r' values)

	Vegetarians (n=23)	Fish Eaters (n=22)
TC	0.237	0.250
HDL – C	-0.242	0.022
LDL – C	0.242	0.084
Non HDL – C	0.401	0.006
TG	0.025	0.207
n = number of subject		

Table 4. Atherogenic index as indicated by various risk ratios

	Vegetarians (n=23)	Fish Eaters (n=22)
TC/HDL-C	4.22 ± 1.63	3.31 ± 1.65
Non HDL-C/HDL-C	2.41 ± 1.60	4.38 ± 1.54
LDL-C/HDL-C	3.40 ± 1.59	2.52 ± 1.64
(TC/HDL-C) x MDA	20.88 ± 14.52	17.22 ± 15.09
(Non HDL-C/HDL-C) x MDA	12.72 ± 12.08	18.04 ± 10.66
(LDL-C/HDL-C) x MDA	14.14 ± 9.56	10.35 ± 8.68
n = number of subjects values are Mean $\pm$ SD		

C and Non HDL-C/HDL-C do not show any significant difference between the vegetarians and fish eaters.

Since increased lipid peroxidation is also a risk factor for CHD, MDA values were multiplied by risk ratios to obtain a new index which serves as a better predictor of CHD. The values obtained using the various risk ratios are presented in Table 4. However, they are not statistically significant.

DISCUSSION

Unlike most studies where fish or fish products were given as a drug, this study investigated the effect of moderate amount of fish in the regular diet on the oxidant status and lipid profile. Plasma MDA that reflects lipid peroxidation has been correlated with serum cholesterol, arterial injury and severity of atherosclerosis. The results of the present study indicate no significant difference in the lipid peroxide levels of vegetarians and fish eaters. This finding is in agreement with the report of Higdon *et al* (22) who studied *in vivo* oxidation by measuring plasma MDA and F2 - isoprostanes. Bhattacharya *et al* (23) have reported increased lipid peroxidation in hyperlipidemia. Ohara *et al* (13) have reported increased superoxide anion production in hypercholesterolemia. Meydani *et al* (24) and Piche *et al* (25) have reported increased concentration of plasma MDA in humans given supplements of fish oil. However, contradictory results about the effects of consumption of eicosapentaenoic acid and docosahexaenoic acid on oxidation were also obtained in *in vivo* studies (26,27,28). The results of the present study give credence to the argument that fish rich diet may not lead to increased oxidation *in vivo*.

Elevated levels of plasma TG have long been associated with the risk of CHD (29,30). In the present study, 60% of vegetarians and 44% of fish eaters showed TG levels above the cut off limit of 160 mg/dl. Lack of significant difference in the serum TG of the two groups is consistent with the literature findings which show that plasma TG are not affected by alterations in saturated, monounsaturated and n-6 PUFAs (31).

HDL-C has an inverse relation to the risk of CHD. Persons with HDL-C levels below 35mg/dL have a CHD incidence rate of more than 8 times, compared to persons with HDL-C levels greater than 65mg/dL or above (32). In our study, though significant difference in HDL-C levels are not observed in the two groups, 39% of vegetarians and only 13% of fish eaters had HDL-C levels less than 40 mg/dl. Interestingly, in the present study, a negative correlation of HDL-C with MDA is seen only in vegetarians. This needs to be explained. Gofman *et al* (33) reported lower HDL-C values in young men aged 20 and above in whom a CHD subsequently developed. Goldbourt *et al* (34) have reported lower HDL-C levels in men aged 40 and above in whom CHD subsequently developed. A hypothetical 1 mg/dl increment in HDL-C is associated with 2.3% decrement of CHD risk (35). However the results of the study do not show a distinct altered lipid status in either group.

Recently the use of non HDL-C has been suggested

as a better index of CHD risk and treatment (21). 35% of the vegetarians and only 27% of the fish eaters had a non-HDL-C greater than 160 mg/dl. A positive correlation of MDA with non HDL-C is seen in vegetarians. However, it is only weakly positive in fish eaters. Similarly the product of the risk ratios and MDA show an overall better lipidemic and oxidant status in fish eaters, compared to the vegetarians.

Lipid profile of an individual depends on many factors. Life style, stress and exercise were not taken into account in the present study. This may be the reason for the lack of significant difference in the lipid profile of vegetarians and fish eaters.

Based on the above findings, it may be reasonable to conclude that lipid peroxidation is not increased in fish eaters as was feared. Our study was not able to clearly establish that consumption of moderate amount of fish in the regular diet improves the lipid status and thereby has a preventive role in CHD. The analysis of the present work was limited by the sample size. A study conducted on a larger scale may be able to throw better light on the above aspect.

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