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Comparative Study of Oxidant Status and Lipid Profile in Vegetarians and Fish Eaters

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ABSTRACT
Atherosclerosis was the leading cause of death and disability in the developed world. Habitual consumption of moderate amount of fish was associated with reduced mortality from coronary heart disease. However the beneficial effects of fish enriched diet seems contradictory due to susceptibility of the Poly Unsaturated Fatty Acid in them to oxidation; it was also acclaimed that vegetarians in general will have low serum cholesterol and a better anti oxidant status than fish eaters. In view of this a comparative study of oxidant status and lipid profile was done in vegetarians and fish eaters. This study enrolled 50 vegetarians and 50 fish eaters of age 35-55 years. Statistical Analysis by the students "T" Test or Mann Whitney "U" Test was applied depending upon the nature of the data. Pearson's correlation co efficiencies (r value) were used to compare the correlations. It showed significant difference in HDL-C levels among fish eaters when compared to vegetarians; this study showed no significant difference in the lipid peroxide levels and the other lipoproteins. The correlation of malondialdehyde with HDL-C was negative in the vegetarians and weakly positive in the fish eaters. LDL-C showed a positive correlation with MDA in both the groups. Triglycerides showed a positive correlation with MDA and correlation was strong in fish eaters.
Keywords= *Atherosclerosis, Lipid profile, Oxidants, Antioxidants, Nutrients in fish.*

INTRODUCTION

Heart Disease is preventable; researchers have been trying for years to find the best ways to reduce the prevalence of it in our society. Besides lifestyle changes, research is in progress for both nutritional and drug treatments. One of these nutrient studies is Omega-3 fatty acids. These long chained polyunsaturated fatty acids are found in high concentration in fish and fish oil and are thought to have preventive effects on heart disease. Studies are therefore done on Omega-3 fatty acid supplements, fish and fish oil and their

possible relations to heart disease. Atherosclerotic disease is the leading cause of mortality in developed countries with Coronary Artery Disease (CAD) being the number one killer of both men and women. In today's world, most deaths are attributable to non communicable diseases (32 million) and just over half of these 16.7 millions are as a result of Cardio Vascular Disease (CVD); more than one third of these deaths occur in middle aged adults. In developed countries, heart diseases and stroke are first and second leading cause of death for adult men and women. Coronary Heart Disease (CHD) is a worldwide disease. In India, an estimate 2.27 million people died due to CVD; during 1990 and according to projections, the number of deaths due to ischemic heart disease (IHD) increased from 1.17 million in 1990 to 1.59 million in 2000 and 2.03 million by 2010. The prevalence of IHD among adults based on clinical and ECG criteria was estimated as 96.7% population in the urban and 27.1% in rural areas.

Epidemiological studies on Greenland Eskimos have shown a correlation between low incidence of CHD and high consumption of fish products[1]. Dietary fiber plays an important role in heart diseases by affecting atherogenesis. It is well established that free radical damage contributes to the etiology of cardiovascular diseases as well as other diseases like Carcinoma, cataract, diabetes etc; there is growing interest in the possible role of free radicals in the development of Coronary Heart Disease. Free radicals can cause oxidation of low density lipoproteins (LDL). Oxidized LDL has been demonstrated to accelerate endothelial damage, macrophage recruitment and increased uptake of LDL-C by foam cells which gradually develop into fatty streaks. Habitual consumption of moderate amount of fish may be associated with reduced mortality from CHD and is attributed to the Omega-3 fatty acids present in them; Bang & Dyeberg[2] emphasized the importance of Omega-3 fatty acids in the prevention of myocardial infarction because of its anti thrombin and hypocholesteremic effect. On the other hand, vegetarians have a greater intake of omega6 series of fatty acids in their diet; Differences in blood lipids between the vegetarians [3] and omnivorous have been well studied. Lipid peroxidation [4] and consequent degradation products such as malondialdehyde (-CHO-CH2-CHO-) are seen in biological fluids there estimation in serum is often employed to assess the oxidant status. Oxidant means the electron acceptor in an oxidation reduction (redox) reaction. The present study aims to estimate the serum malondialdehyde (MDA) by Thio Barbiburic Acid (TBA) calorimetric assay. Free radical theory [5] and its importance to disease and aging was initially articulated in 1954 by Denham and Harman [6] Chemical oxidation by free electrons damages cell structures such as mitochondria, cell membranes, enzymes and DNA as the damage accumulates degeneration of these structures leads to diseases that are responsible for up to 90% of deaths.

The following are the free radicals in the living cells - Superoxide anion (O_2^-) the partially reduced hydrogen peroxide (H_2O_2) and hydroxyl free radical (OH*). Of these intermediaries in reduction of O_2 to water, the hydroxyl radical is undoubtedly the most dangerous free radical as it is involved in reactors such as lipid per oxidation and generation of other toxic radicals. Hydrogen peroxide itself is not a free radical but is converted by the Fenton or Haber-Weiss reactions to the hydroxyl radical in the presence of Fe^{2+} or calcium prevalent in cells. Reactive oxygen species(ROS) mediated oxidative damage macromolecules namely lipids, proteins and DNA have been implicated in the pathogenecity of major diseases such as cancer [7], rheumatoid arthritis, post ischemic reperfusion injury, myocardial infarction, cardiovascular disease etc [8]. Fish is an excellent source of complete proteins, Lipids, phosphorous, Iron, and other minerals, some are high in vitamin A and fish is a rich source of Omega-3 fatty acids. Sea fish contains Iodine; liver of white fish contains vitamins A, D & E. Eating fish three times a week

has been associated with a significant decrease in the rate of heart disease; this became apparent when Scientists noted that coronary artery disease- a leading cause of death in North America – was almost nonexistent among the indigenous people of Greenland, Japanese fisherman, and first nation of the Pacific Northwest. The one factor that these three groups had in common was a diet that relied heavily on fish for proteins. When researchers looked at the effects of diets in other population they found that men who ate fish regularly two or three times a week was much less likely to suffer Heart attacks than others. In view of the present scenario, this work was taken up to study the oxidant status and lipid profile in vegetarians, fish eaters.

MATERIALS AND METHODS

This study was conducted on 100 subjects out of them 50 were vegetarians, 50 were predominantly fish eating people in the age group of 35-55 yrs. The study was approved by the Institutional ethics committee. Written informed consent was taken from the patients in local language. This is an observational and cross sectional study. Age, height, weight and blood pressure were recorded and food habits of the subjects were obtained using a questionnaire

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 meals (approximately quality was calculated).

Exclusion Criteria

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

Collection of Blood Samples

5ml of heparin zed venous blood was collected after an overnight fast of 12 hrs and the following parameters were estimated in the plasma with in 2 hrs of sample collection. Serum was separated and estimation of__total cholesterol by CHOD-PHOD/phospho tungstate method of Allianc [9], estimation of High Density Lipoprotein (HDL) cholesterol by CHOD – POD phosphotungstate method by Burstein [10], estimation of triglycerides by glycero phosphate oxidase method of jacobe [11], estimation of LDL by fride wald equation [12]: LDL cholesterol = cholesterol – (HDL + VLDL).

And estimation of MDA levels (Malondialdehyde) by Thiobarbituric Acid (TBA) reaction for MDA (Mahalouz et al 1978) was used. Principle: Under the acidic and the treating conditions of the reaction the lipid peroxides breakdown to form Malondialdehyde which complexes with Thiobarbitutic acid (TBA).The resulting MDA-TBA chromogen is measured at 530nm distilled against distilled water, in a spectrophotometer

Body Mass Index (B.M.I)[13] was calculated in both men and women as follows: BMI = Weight in Kg/Height in (meters) ^{2}

Statistical Analysis by the students "T" Test or Mann Whitney "U" Test was applied depending upon the nature of the data. Pearson's correlation co efficiencies (r value) were used to compare the correlations.

RESULTS

The study was initiated in August 2012 and completed in July 2013 at Government General Hospital and Siddhartha medical college, Vijayawada, Andhra Pradesh, India.

Plasma lipid peroxides and Lipid profile in Vegetarians and Fish Eaters were shown in table no 1:

Sl.No	Parameters	Vegetarians (n=50)	Fish Eaters (n=50)
1	MDA (m mols/L)	202±36.6	210±31.41
2	Total Cholesterol (mg/dl)	182.5±19.32	188.56±25.01
3	HDL –C (mg/dl)	44.3±6.94	60.84±16.69
4	LDL-C (mg/dl)	108.46±20.48	102.78±15.44
5	VLDL-C (mg/dl)	27.7±10.84	24.35±7.52
6	Triglycerides (mg/dl)	92.6±43.39	121.84±33.19

n = No of subjects, Values are mean ± standard deviation, MDA= malondialdehyde, HDL-C= high density lipoprotein, LDL-C= low density lipoprotein, VLDL-C= very low density lipoprotein.

The mean content of Plasma MDA is 202 ± 63.62 in vegetarians and 210 mmol/L ± 31.41 in fish eaters the difference between the two groups was not significant. The mean of HDL cholesterol and Triglycerides were increased in case of fish eaters.

Other lipid parameters cholesterol, LDL, VLDL did not showed any significant difference between vegetarians and fish eaters; all the values in both the groups were well within the normal limits.

The r values for the correlation of MDA with lipid parameters were given in table no 2.

S.No.	Parameters	Vegetarians (n=50)	Fish Eaters
1	Total Cholesterol(mg/dl)	0.373	0.088
2	HDL-C(mg/dl	-0.507	+0.884
3	LDL-C (mg/dl)	0.128	0.191
4	VLDL-C (mg/dl)	0.229	0.656
5	Triglycerides (mg/dl)	0.034	0.592

n=number of subjects, HDL-C= high density lipoprotein, LDL-C= low density lipoprotein, VLDL-C= very low density lipoprotein.

The correlation of MDA with HDL-C was negative in the vegetarians (r= –0.507) and weakly positive in the fish eaters (+ 0.884). LDL-C showed a positive correlation with MDA in both the groups. Triglycerides showed a positive correlation with MDA and correlation was stronger in Fish eaters (r=0.592 Vs 0.034).

Atherogenic index was indicated by various risk factors were shown in table no 3.

Sl.No	Parameters	Vegetarians (n=50)	Fish Eaters (n=50)
1	T. C\HDL-C	4.11±2.7	2.00±1.5
2	LDL/HDL	2.44±3.33	1.68±1.08
3	TC/HDL X MDA	832.16±1.10	420.55±20.6
4	LDL/HDL X MDA	494.55±13.33	354.64±28.7

n= Number of Subjects, Values are Mean ± standard deviation, T.C= total cholesterol, MDA= Malondialdehyde.

TC/HDL-C ratio was higher in vegetarians when compared to fish eaters. The ratio of LDL/HDL was also a little higher in vegetarians in comparison to fish eaters. Since increased lipid per oxidation was 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 however they were not statistically significant. The relationship between cholesterol and Atherosclerotic coronary disease was not linear, if a risk ratio of 1.0 is assigned at the cholesterol value of 200 mg/dl (5.18 m

mol/L); the risk ratio increases to 2.0 at 250mg/dl (6.48 m mol/L); and 4.0 at 300 mg/dl (7.77 m mol/L) 30. Total Cholesterol by HDL ratio more than 3.5 was dangerous. Similarly, LDL-HDL ratio more than 3.5 was also deleterious.

DISCUSSION

This study investigated the effect of moderate amount of fish in regular diet on the oxidant status and lipid profile. The results of this study indicate little or no significant difference in the lipid peroxide levels of vegetarians and fish eaters. This finding was in agreement with the report Higdon Et al [14] who studied in vivo oxidation by measuring plasma MDA and PGF2 – Isoprotanes. Bhattacharya Et al [15] had reported increased lipid per oxidation in hyper lipidemia. Ohara Et al [16] had reported increased superoxide anion production in hypercholesterolemia. Meydane Et al [17] and Piche [18] Et al had reported increased concentration of plasma MDA in humans given supplements of fish oil. The results of this present study gave credence to the argument that fish diet may not lead to increased oxidation in vivo.

The W-$_3$ (Omega) Poly Unsaturated Fatty Acid (PUFA) had a unique physiological effect that may decrease the risk of heart disease: they decrease platelet aggregation and were thought to act by one of tromboxane A_3, which is only weakly proaggregating, and prostaglandin I_3 (PGI), which is strongly antiaggregation would be shifted towards a more antiaggregating condition. The W-$_3$ (Omega) Poly Unsaturated Fatty Acid (PUFAs) may also act by simply inhibiting the conversion of arachidonic acid to TAX_2. Elevated levels of plasma Triglycerides (TG) had long been associated with the risk of Coronary Heart Disease (CHD); in this present study 46% of vegetarians and 60% of the fish eaters showed Triglycerides (TG) levels below the cutoff point 150 mg/dl. Lack of significant difference in the serum Triglycerides (TG) of the study groups was consistent with the literature finding which showed that plasma Triglycerides (TG) were not affected by alterations in the saturated, monounsaturated and n-6 Poly Unsaturated Fatty Acid (PUFA)

In this study significant difference was observed between the fish eaters and vegetarians, in 22% of vegetarians HDL-C levels were less than 50 mg/dl. 30% of vegetarians had HDL-C levels more than 50 mg/dl. Interestingly in this study a negative correlation of HDL-C with MDA was seen only in Vegetarians.

CONCLUSIONS

The best proven way to prevent the onset of heart diseases is through life style modification. The risk of developing heart disease can be prevented in many ways. It could be through the change of habits in their own lifestyles. Exercising regularly, eating foods low in fat and salt, no smoking and deleting stress from our lives are some of the primary ways of keeping a healthy heart. However, eating fish is a healthy thing to do, regardless of whether you are getting a hefty dose of Omega -3 fatty acids. Recent research suggest that eating fish helps to prevent heart attacks, possibly because if you were eating fish you were not eating red meat, which was high in saturated fat. As with many of the illnesses that plague our society, several drugs and treatments have been discovered in order to address the problem once it is already implanted in a person. Some of these treatments are drugs that help to lower the cholesterol level in the blood while others are nutritional treatments that naturally help to reduce the risk of getting heart diseases.

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