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Original Article

Influence of Fish Consumption on Oxidant Status and High Sensitive- C - Reactive Protein: A Comparative Study among Fish Consuming and Vegetarian Subjects

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ABSTRACT

Background: Cardiovascular disease (CVD) is the most frequent cause of morbidity and mortality in India. Reduced incidence of CVD was observed in populations with a high or moderate fish consumption due to the long-chain n-3 polyunsaturated fatty acids (PUFA) present in fish oils, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids inhibit the pro-inflammatory process which reduces the production of inflammatory mediators. The initiation, growth, and complications of atherosclerosis are actually inflammatory responses that elevate CRP levels in the blood. Due to the susceptibility of PUFA to oxidation, the beneficial effect of fish diet seems to be contradictory. It was aimed to evaluate whether the habitual consumption of moderate amount of fish is associated with changes in the level of oxidant status and C-reactive protein (hs-CRP) in fish consuming adult subjects. **Materials and Methods:** The study subjects comprised of 25-40 year aged healthy 150 vegetarians who were consuming purely lacto-vegetarian diet and 150 individuals who were taking ≥ 5 times fish meal in a week along with vegetables and occasionally other meat. Oxidant status in erythrocyte suspension was measured by assessing the level of malondialdehyde (MDA) at the end of 0h and at the end of 2h using hydrogen peroxide as an oxidizing agent. Serum hs-CRP was measured by latex enhanced immunoturbidometric assay. **Results**: The mean level of hs-CRP was lower in fish consuming subjects ($p<0.05$) when compared to the vegetarian subjects and the oxidant status does not vary significantly between the two groups. **Conclusion:** The present study findings clearly project that habitual consumption of moderate amount of fish is associated with lowering of the inflammatory marker and fish rich diet has not increased oxidation status in fish consuming subjects.

Key words: Fish eaters, Vegetarians, MDA, hs-CRP, Cardiovascular disease.

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1. INTRODUCTION

Non communicable diseases are the leading cause of death in the world. In India 53% of the death rate due to non-communicable disease, in which 24% contributed by cardiovascular disease (CVD), 11% due to chronic respiratory disease, 6% from cancer and 2% from diabetes. Increased CVD prevalence rate observed in Asian Indian population. Prevalence of CVD risk factors found higher in urban population and it is also increasing in rural population. Since the majority of the Indians live in rural area, the health care education is beneficial in prevention, detection in earlier stage and management of CVD[1,2]. CVD mortality is increasing due to major socioeconomic changes, change in lifestyle, increasing stress levels, increasing intake of saturated fats and tobacco, demographic changes, rapid industrialization and urbanization[3-5]. The individuals from lower socioeconomic backgrounds frequently do not receive optimal therapy which increases the CVD mortality rate[6].The overall prevalence of Coronary Artery Disease (CAD) in South Indian population is 11% and males in the age group of 20-29 years are suffering from CAD which indicates the existence of CVD in younger age population[7].

Atherosclerosis is characterized by a complex multifactorial pathophysiology. Inflammation plays an important role in all stages of atherosclerosis. High sensitivity C-reactive protein (hs-CRP) is an acute-phase inflammatory mediator mainly regulated by interleukin (IL)-6 in the liver and has been shown to predict future CVD events[8,9].

The epidemiological studies observed inverse correlation between consumption of dietary fish or fish oil and biomarkers of inflammation and the ratio of n-6 to n-3 PUFA play significant roles in modulating hs-CRP concentrations and other markers of inflammation[10]. Several studies suggest that omega-3 fatty acids are cardio-protective, have anti-inflammatory properties, reduce hypertension, inhibit thrombosis and arrhythmia and improve arterial compliance[11-16].

Dietary fat plays an important role in heart diseases by affecting atherogenesis. Habitual consumption of moderate amount of fish may be associated with reduced mortality from CHD and is due to the long chain ω-3 Polyunsaturated fatty acids (PUFA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) present in them. Epidemiological studies on Greenland Eskimos have shown a correlation between low CHD and high consumption of fish products. However, fish enriched diet is susceptible to oxidation due to the presence of PUFAs in them[17].

Cells can tolerate mild oxidative stress and restores the balance with help of antioxidant defense system. But when severe, cause derangement in all metabolisms causing cell injury and death. Oxidative stress plays a role in various clinical conditions such as malignant diseases, diabetes, and atherosclerosis. RBC's are most vulnerable to oxidative stress which causes lipid peroxidation leading to hemolysis. Determination of malondialdehyde (MDA) using thiobarbutric acid is used to measure the extent of lipid peroxidation in RBC suspension[18-19].

Thus, an attempt has been made to compare the plasma hs-CRP level and the erythrocyte MDA level in fish eaters and vegetarians and to evaluate the correlation between plasma hs-CRP and erythrocyte MDA in fish eaters and vegetarians.

2. MATERIALS AND METHODS

Selection of subjects: In this population-based comparative study, 150 vegetarians and150 predominantly fish eating subjects were taken from some of the selected areas of coastal region of Dakshina Kannada, Karnataka. The age group of 25-40 years was chosen for the study.

Inclusion criteria: Vegetarians: Subjects consuming purely lacto vegetarian diet.

Fish Eaters: Subjects consuming ≥ 5 times fish meal in a week, fish along with vegetables and occasionally other meats (An approximate quantity was calculated by dividing the quantity of fish cooked by the number of family members consuming it).

Exclusion criteria: The following individuals were excluded from the study.

Diabetes mellitus, hypertension, pregnancy, smoking, chronic kidney diseases, those suffering from chronic inflammatory diseases like tuberculosis, leprosy etc.

An ethical clearance was obtainedfrom the institutional ethics committee. An informed consent was taken from the subjects. Age, height, weight and waist circumference were recorded and dietary profile of the subjects were obtained using a questionnaire.

Sample collection: Five ml of venous blood was collected after an overnight fast of 8-10 hours. The blood was collected in plain tube for the estimation of (high sensitive) hs-CRP and in EDTA tube for the estimation of oxidant status in the erythrocyte suspension.

Oxidant status in erythrocyte suspension was measured as thiobarbituric acid reactive substance, malondialdehyde (MDA) at the end of 0 hour and at the end of 2hour using hydrogen peroxideas oxidizing agent[20,21]. Serum hs-CRP was measured by latex enhanced immune-turbidometric assay on fully automated analyser Biolis 24i[22].

Statistical analysis: The parameters analysed were expressed as mean± SD. To analyse the difference among the parameters unpaired t-test was used and associations were analysed by calculation of correlation (r-value) using SPSS program. The $p<0.05$ was considered to be significant.

3. RESULTS

Anthropometric data of two groups are presented in Table1. Total 150 predominantly fish eating subjects and 150 vegetarians having comparable age, sex, BMI and WC were enrolled for the study. The value of inflammatory marker hs-CRP in the plasma and the oxidative stress assessed by estimating MDA in RBC suspension are presented in Table

2. The mean±SD value of MDA at the end of 0 hour is 3.83 ± 0.53 (µ mol/L) in fish eaters and 3.72 ± 0.60 (µ mol/L) in vegetarians. The difference between the two groups is not statistically significant. And also the mean±SD value of MDA measured at the end of 2 hours using hydrogen peroxide as an oxidizing agent is 6.03 ± 0.7(µ mol/L) in fish eaters and 5.92 ± 0.65(µ mol/L) in vegetarians. The difference between the two groups is not statistically significant. The value of inflammatory marker hs-CRP is lower in fish eaters 0.75 ± 0.36 (mg/L) compared to vegetarians 0.84 ± 0.33 (mg/L) and the statistically significant difference was observed between the two groups.

The 'r' values for the correlation of hs-CRP with MDA level are given in Table 3. Although correlation of hs-CRP with MDA (0h) is weakly negative in both the groups, association was stronger in fish eaters compared to vegetarians. hs-CRP with MDA (2h) showed weakly negative correlation in fish eaters and weakly positive correlation in vegetarians.

Table 1: Anthropometric data

S.No.	Parameters	Fish eaters (n=150)	Vegetarians (n=150)
1.	Age (years)	32.9±4.7	33.5±5.3
2.	BMI (Kg/m^2)	25.9±4.5	24.2±4.5
3.	WC (cm)	83.2±12.1	85.1±11.3

n = number of subjects Values are expressed as Mean± SD
BMI=Body mass index
WC=Waist circumference

Table 2: Oxidant status and inflammatory marker of the study groups

Parameters	Fish eaters (n=150)	Vegetarians (n=150)
MDA^a (µ mol/L)	3.83 ± 0.53	3.72 ± 0.60
MDA^b (µ mol/L)	6.03 ± 0.79	5.92 ± 0.65
hs-CRP (mg/L)	0.75 ± 0.36	0.84 ± 0.33*

MDA^a =MDA measured at the end of 0h. Values are expressed as Mean± SD
MDA^b= MDA measured at the end of 2h.
hs-CRP= High sensitive C-reactive protein. *p<0.05

Table 3: Correlation of hs-CRP with the MDA values of the study groups ('r' values)

Parameters	Fish eaters (n=150)	Vegetarians (n=150)
MDA 0 h	-0.090	-0.009
MDA 2 h	-0.053	0.015

n= number of subjects

4. DISCUSSION

This study investigated the effect of moderate amount of fish in the regular diet on the inflammation and oxidative stress in RBCs. The results of the present study showed significant difference in the inflammatory marker, hs-CRP and no significant difference in the lipid peroxide levels in RBCs of fish eaters and vegetarians.

The result obtained on inflammatory marker is in agreement with the report of Micallef *et al.,* who studied inverse relationship between plasma n-3 fatty acids and C- reactive protein[23]. Mazidi *et al.,* reported the role of dietary PUFAs in decreasing hs-CRP levels in US adults[24]. The study conducted on a group of healthy adults by Kalogeropoulos *et al.,* in which N-3 fatty acid levels assessed in blood were inversely associated with CRP[25]. The study conducted by Khosroshahi on patients of haemodialysis, CRP was not lowered after n-3 supplementation (2.4 g/day) for a small group of haemodialysis patients[26]. The result obtained on oxidative stress in RBCs is in agreement with the report of Cariappa *et al.,* who reported no change in the concentration of plasma MDA[17]. The study of Mabile *et al* also reported stable erythrocyte resistance to oxidative stress in hypertriglyceridemia patients[27]. The study on diabetic patients conducted by Nandini *et al.,*reported erythrocyte membrane susceptibility to peroxidation which is not altered in diabetic patients[28]. However, contradictory results about the effects of consumption of eicosapentaenoic acid and docosahexaenoic acid on oxidation were also obtained in *in vivo* study[29]. The results of the study have not supported the argument that fish rich diet may lead to increased oxidation *in vivo*.

5. CONCLUSION

On the basis of the data in this study, it can be suggested that inflammatory marker hs-CRP is lowered in fish eaters and lipid peroxidation is not increased in them. The outcome of the study reveals that consumption of moderate amount of fish in the regular diet decreases the inflammatory marker without affecting the oxidant status and thereby has a preventive role in CHD. A study conducted on a larger scale may be able to provide better results on the above aspect.

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