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ORIGINAL ARTICLE

Dietary Fats and Oxidative Stress: A Cross-Sectional Study Among Coronary Artery Disease Subjects Consuming Coconut Oil/Sunflower Oil

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Abstract Coconut oil has been used by the people of Kerala as a cooking medium for several decades. Due to its alleged hypercholesterolemic activity, general population in recent times is shifting to cooking oils rich in polyunsaturated fats, the most popular being sunflower oil. The effect of long-term consumption of sunflower oil on oxidative stress in humans is not well investigated. We studied oxidative stress among coronary artery disease (CAD) patients who were consuming coconut oil or sunflower oil as a part of their routine diet. Men, aged 35–70 years, with established CAD, who presented to the hospital for routine cardiac evaluations, were enrolled in this observational study. Group 1 and 2 consisted of 73 and 80 subjects consuming coconut oil and sunflower oil respectively for over a period of 2 years. Lipid profile and parameters for oxidative stress were evaluated among them. Conventional lipid parameters did not differ significantly between the two groups. Mean vitamin C concentration was significantly reduced for subjects on sunflower oil compared to those consuming coconut oil ($P = 0.044$). Malondialdehyde was higher for sunflower oil consumers compared to coconut oil consumers ($P < 0.0001$). Other parameters such as oxidized LDL, GSH, GPx and SOD were not found to be significantly different between the two groups. The results of the present study show that coconut oil did not induce hypercholesterolemia compared to sunflower oil. On the other hand, sunflower oil group had elevated oxidative stress compared to coconut oil group.

Keywords Dietary oils · Routine consumption · Lipid peroxidation · Oxidative stress · Lipid profile

Introduction

Coronary artery disease (CAD) is a major socio-economic burden in India, affecting approximately 7% of the rural and 13% of the urban population [1]. Kerala is a small state located at south-western part of India with a population of approximately 34 million in 2012. Kerala has high literacy rate and has made enviable progress towards health indicators such as life expectancy and infant mortality compared to the rest of India [2]. But community-based studies show that the prevalence of risk factors for CAD such as hypertension, hypercholesterolemia and diabetes mellitus in this region is quite high, and is comparable with modernized, Western populations [2]. Though large-scale studies on the prevalence of CAD from this region are lacking, results of a community-based cross-sectional study published in 2016 has shown that the overall crude and age-adjusted prevalence of any CAD in Kerala was 12.5% [3].

Coconut oil has been used by people of Kerala as cooking medium for several decades. As coconut oil is rich in saturated fats, it has been proposed to be a major contributor to the rise in the incidence of CAD in this region. Hence, general population is shifting to cooking oils rich in polyunsaturated fats like sunflower oil over the past few decades. There is significant controversy on the association of coconut oil with CAD. Saturated fatty acids of coconut oil, being primarily medium-chain fatty acids, do not need

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esterification in liver and are considered not to induce hyperchelosterolemia [4]. But various studies have reported results contradicting this hypothesis [5]. Studies on the effect of coconut oil on antioxidant defense system have mostly been done using animal models and have compared saturated, polyunsaturated and monounsaturated fats [6, 7]. Though the cholesterol-lowering capability of sunflower oil has been well documented, it is not known if long-term consumption of sunflower oil as a part of routine diet affects oxidative stress in humans. Hence, we conducted a study to compare the lipid profile and selected indicators for oxidative stress among CAD subjects who were consuming coconut oil with those consuming sunflower oil as a part of their routine diet.

Materials and Methods

The study was conducted at a tertiary care University hospital in Kerala. Institutional Ethics Committee approved the protocol and the study was conducted according to Declaration of Helsinki and its ensuing amendments. Informed consent was obtained from all the participants. We considered men, aged 35–70 years, for enrolment into the study. Patients who visited the Cardiology OP for routine check-up were screened. Subjects with previously documented myocardial infarction or angiographically proven CAD were included in the study. Group 1 and 2 consisted of 73 and 80 subjects consuming coconut oil and sunflower oil respectively. Only those subjects who were consuming the respective oil as the predominant source of fat for over a period of 2 years were included. The subjects were interviewed during their recruitment and the details regarding their diet and life style habits were collected and entered in a proforma. Details regarding the severity of CAD and medication of the enrolled subjects were procured from the case sheets and hospital information software. The subjects in each group were divided into three sub-groups based on the severity of CAD: single vessel disease, double vessel disease and triple vessel disease. The corresponding sub-groups of coconut oil group and sunflower oil group were compared.

Fasting blood sample was collected from each subject. Total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were analyzed (direct method) with Hitachi 912 autoanalyzer using kits from Roche Diagnostics, Mannheim, Germany. Apo B in serum was analyzed by immunoturbidimetry using kit from Daiichi Chemical Co., Japan. Vitamin C was estimated in plasma by dinitrophenyl hydrazine assay [8]. Oxidized LDL in plasma was estimated using Mercodia Oxidized LDL ELISA kit (Mercodia, Sweden) based on direct sandwich ELISA technique. Superoxide dismutase (SOD) in serum was estimated based on the method of Marklund and Marklund, and as modified by Nandi and Chatterjee [9, 10]. Erythrocytes were washed thrice in cold saline, lysed and used for the determination of malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GPx). GSH was estimated using the method of Beutler et al. [11]. GPx was assayed according to Paglia and Valentine, and as modified by Lawrence and Burk [12, 13]. MDA was estimated by the method of Jain et al. [14].

Categorical variables were expressed as percentages and continuous variables were expressed as mean ± standard deviation. Chi square test was used to compare categorical variables and Student's "t" test was used to compare continuous variables. A P value of ≤ 0.05 was considered to be statistically significant. Correlation between the variables was checked by Pearson correlation analysis. Statistical analysis of data was done using SPSS, version 16.0.

Results

Clinical characteristics of the study subjects are represented in Table 1. All the enrolled subjects were on cholesterol-lowering therapy and hypertensives/diabetics among them were on antihypertensive and/or anti-diabetic medications. Age, percentage of diabetics, hypertensives, smokers etc. were not significantly different between the two groups. A current tobacco user in this study is defined as someone smoking cigarettes/beedis or using smokeless tobacco within past 3 months of recruitment. We did not observe significant difference between the two groups on comparing the frequencies of patients with single vessel, double vessel or triple vessel disease. The subjects were following a typical Kerala diet with carbohydrate forming the predominant calorie contributor. The subjects derived approximately 13–20% of their daily calories from the oil, and this was not significantly different between the two groups.

Results of comparison of the biochemical parameters of the two groups are shown in Table 2. Total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol did not show significant difference between the two groups. Though triglycerides and HDL-cholesterol was lower among those using sunflower oil, the results did not attain statistical significance. Apo B was not found to be significantly different between the two groups ($P = 0.253$). High standard deviation observed for lipid parameters might have contributed to the non-significant results obtained while comparing these parameters. Mean vitamin C concentration was significantly reduced for subjects on sunflower oil compared to those consuming coconut oil ($P = 0.044$). Malondialdehyde was higher for sunflower oil consumers compared to coconut oil consumers

Table 1 Comparison of the clinical profile of the subjects of the two groups

Variables	Group 1 (Coconut oil)	Group 2 (Sunflower oil)	*P* value
Mean age (years)	59.8 ± 4.54	53.4 ± 7.23	1.0
Smokers, n (%)	10 (13.7%)	8 (10%)	0.478
Hypertensives, n (%)	47 (64.3%)	42 (52.5%)	0.066
Diabetics, n (%)	38 (52.1%)	41 (51.3%)	0.799
Single vessel disease, n (%)	25 (34.2%)	30 (37.5%)	0.675
Double vessel disease, n (%)	34 (46.6%)	29 (36.3%)	0.537
Triple vessel disease, n (%)	14 (19.2%)	21 (26.2%)	0.298

Table 2 Comparison of biochemical parameters between the two groups represented as Mean ± SD

Variables	Group 1 (Coconut oil)	Group 2 (Sunflower oil)	*P* value
Total cholesterol (mg/dL)	148.24 ± 36.54	147.16 ± 26.7	0.419
Triglycerides (mg/dL)	119.2 ± 49.49	107.78 ± 44.05	0.099
HDL-cholesterol (mg/dL)	41.3 ± 18.93	37.09 ± 9.84	0.077
LDL-cholesterol (mg/dL)	96.71 ± 30.59	93.11 ± 22.06	0.197
Apo B (mg/dL)	92.41 ± 17.97	90.12 ± 21.73	0.253
Vitamin C (mg/L)	9.05 ± 1.66	8.56 ± 1.89	0.044
GSH (nmoles/gHb)	5.25 ± 0.93	5.3 ± 0.84	0.368
GPx (IU/gHb)	14.71 ± 3.51	14.61 ± 2.3	0.403
SOD (U/mL serum)	4.79 ± 0.85	4.92 ± 1.04	0.216
MDA (nmoles/gHb)	16.84 ± 0.88	17.65 ± 0.95	0.0001
Oxidized LDL (U/L)	77.18 ± 31.55	75.94 ± 29.08	0.33

SD standard deviation, *mg/dL* milligram per decilitre, *nmoles/g Hb* nanomoles per gram hemoglobin, *IU/g Hb* international units per gram hemoglobin, *U/mL* units per millilitre, *mg/L* milligram/litre, *U/L* units per litre

Table 3 *P* value for oxidative stress parameters on comparing the sub-groups using coconut oil with sub-groups using sunflower oil

Variables	Single vessel disease	Double vessel disease	Triple vessel disease
Vitamin C	0.438	0.073	0.041
GSH	0.945	0.986	0.327
GPx	0.636	0.919	0.874
SOD	0.212	0.986	0.832
MDA	0.039	0.0001	0.0001
Oxidized LDL	0.703	0.799	0.825

($P < 0.0001$). Other parameters such as oxidized LDL, GSH, GPx and SOD were not found to be significantly different between the two groups.

Results of sub-group analysis based on the severity of CAD are given in Table 3. When subjects with single vessel disease were compared between two groups, it was found that MDA was significantly higher among subjects on sunflower oil ($P = 0.039$). A similar result was observed for subjects with double vessel and triple vessel disease using sunflower oil compared with those using coconut oil ($P = 0.0001$ for both sub-groups). Vitamin C concentration was found to be lower among subjects with triple vessel disease using sunflower oil compared to those with triple vessel disease using coconut oil ($P = 0.041$).

Pearson correlation analysis of the parameters measured for oxidative stress showed that the variables were not significantly correlated except for a mild positive correlation between MDA and oxidized LDL ($r = 0.155$) and inverse correlation between MDA and vitamin C concentrations ($r = -0.132$).

Discussion

Coconut oil forms the cooking medium of a vast majority of the Kerala population. Due to its high saturated fat content, a considerable proportion of this population has replaced coconut oil with sunflower oil as their cooking medium. We undertook this study to find any significant

differences in oxidative stress between CAD patients consuming these two dietary fats. Impaired serum cholesterol has been established as a major risk factor for CAD by many major studies [15]. Though there have been many studies that have investigated the impact of coconut oil on lipid parameters, the results obtained were not consistent [5, 16]. In our study, no noticeable differences were found between the two groups for conventional lipid parameters. We observed that the contribution of the dietary oils to the daily calorie intake among our study subjects was less compared to studies in Western populations (<20 vs. 35%) that reported the hypercholesterolemic potential of coconut oil [5]. A recent randomized, follow-up study conducted in Kerala population showed that lipid profile did not differ significantly between subjects who consumed coconut oil or sunflower oil during the entire follow-up period [17]. Lipid profile of CAD subjects in this study had been regulated therapeutically and this may be also a reason for the insignificant results obtained for these parameters. Apo B concentration, which represents the LDL particle number, was also not found to be considerably different between the two groups in the present study. There have been studies that showed that apo B concentrations did not vary significantly between healthy men on diets rich in saturated fat or n-6 fatty acid [18].

Atherosclerosis is a progressive inflammatory disease and oxidative stress plays a pivotal role in all stages of its pathogenesis. Generation of lipid peroxides lead to formation of oxidatively modified LDL, that in turn, upregulates the key mediators of atherosclerosis. It is hypothesized that dietary fats influence the susceptibility of cells to oxidative stress by inducing changes in cell membrane fatty acid composition [19]. Various studies that investigated the effect of feeding unsaturated fat in animals have shown that highly unsaturated fats are prone to induce membrane lipid peroxidation [6, 20]. Though such studies are limited among human subjects, available literature shows that sunflower oil could cause an increase in lipid peroxidation when compared to monounsaturated fat rich rapeseed oil [21]. In the present study, the rate of lipid peroxidation was found to be higher for sunflower oil consumers compared to coconut oil consumers. This result was significant even while the subjects were categorized based on the severity of CAD, but there is little literature available to draw a proper comparison. LDL particles rich in polyunsaturated fats are more readily oxidized, and this could have contributed to the increased rate of lipid peroxidation observed among the sunflower oil group subjects. It has been reported that the higher atherogenic risk owing to elevated lipid peroxidation rate on consuming a polyunsaturated fat-rich diet is compensated by its capability to reduce LDL concentration [22], though sunflower oil consumers in our study did not show clinically significant reduction in LDL concentrations compared to coconut oil group. Oxidatively modified LDL is formed when polyunsaturated fatty acids present in the LDL undergoes oxidation. In spite of higher lipid peroxidation rate, an expected similar rise in oxidized LDL was not seen among subjects on sunflower oil compared to those on coconut oil in the present study. Investigators have shown in an in vitro study that oxidation of LDL was similar for saturated fat compared with ω-6 polyunsaturated fat, while it was lower for monounsaturated fat [23].

Antioxidants derived from diet or produced endogenously facilitate in quenching excess free radicals formed in our body. Vitamin C is a chain breaking antioxidant that inhibits lipid peroxidation chain reaction and LDL oxidation. Reduced vitamin C concentration has been reported in CAD subjects compared to normal subjects previously [24]. Though some studies support the role of vitamin C in reducing lipidaemia, the cardioprotective effect of vitamin C on supplementation is yet to be proved conclusively [25]. Our study shows that vitamin C levels were lower among sunflower oil group compared to coconut oil group, but a comparison with similar human studies could not be made due to lack of such studies. A study in New Zealand white rabbits from our group observed that vitamin C levels did not differ between animals fed coconut oil or sunflower oil [6]. Another study in Wistar rats showed that vitamin C levels did not differ significantly between animals fed sunflower oil or cocoa butter rich in saturated fat [26].

SOD, GPx, catalase (CAT) and GSH form the enzymatic antioxidant system in animals. Investigators have reported a reduction in the activity of these antioxidant enzymes among CAD patients, which facilitates the oxidative aggression of the cells. A study showed that while SOD and CAT levels are increased to protect and prevent lipid peroxidation in the early stages of CAD, their levels decreased significantly with the worsening of the disease [27]. A prospective study among 504 patients showed that red blood cell GPx-1 activity is associated with increased cardiovascular risk according to the extent of atherosclerosis [28]. Our study showed that the levels of antioxidant enzymes evaluated, viz. SOD, GSH and GPx were similar in both groups. This is in agreement with a prospective study in the same population that showed that antioxidant enzymes did not differ significantly between CAD subjects on coconut oil or sunflower oil throughout the follow-up period [17]. A study among type 2 diabetic patients showed that antioxidant enzymes did not differ between groups consuming either of the oils [29]. On the other hand, a Mediterranean diet rich in monounsaturated fat may exert beneficial effects in regulating oxidative stress by improving the antioxidant enzyme concentrations. A randomized, interventional study had concluded that monounsaturated fats rich diet improved postprandial GSH

levels and lowered SOD levels indicating reduction in oxidative stress compared to high saturated fat diet [30].

Though this study has produced some interesting results, there are few important limitations for this study. This observational study done in a small population was not conducted under controlled conditions. Medications for lipid lowering, diabetes and hypertension have been shown to have profound effects on antioxidant status and this might have affected our study results. The intra and intergroup differences in diet composition, life style and physical activity might also have influenced the study results.

Conclusions

The results of the present study show that the subjects consuming coconut oil did not demonstrate hypercholesterolemia compared to the sunflower oil group. Meanwhile, the subjects in the sunflower oil group had elevated oxidative stress as indicated by their lower vitamin C levels and higher rate of lipid peroxidation. Thus, the antioxidant status of the subjects consuming coconut oil in this study was better compared to those consuming sunflower oil.

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