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ATHEROTHROMBOTIC RISK FACTORS AND PREMATURE CORONARY HEART DISEASE IN NAGAPATTINAM DISTRICT, INDIA: A CASE-CONTROL STUDY METHODS

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ABSTRACT

Coronary heart disease (CHD) is epidemic in India characterized by premature onset and high mortality. This study shows that premature coronary artery disease in Indians is due to combination of thrombosis and atherosclerotic vascular risk factors. These risk factors are highly prevalent in the community. Prevention and control of premature cardiovascular diseases in India needs urgent control of these factors. Improving lifestyles with tobacco cessation, diet modulation with more fruits and vegetables and lower fat intake, and increased physical activity are critical. Target oriented control of hypertension, lipid levels and glycaemia is required. The study adds that lowering fibrinogen and homocysteine using novel strategies as well as control of all the risk factors from an early age are essential to prevent premature disease.

INTRODUCTION

Coronary heart disease (CHD) is epidemic in India characterized by premature onset and high mortality. The World Health Organization (WHO) reports that while more than 70 per cent of coronary deaths occur in subjects older than 70 yr in North America and Western Europe, in India and other developing [2] countries 70 per cent deaths occur in subjects less than 70 yr of age[1] Factors of risk for the premature CHD in Indian subjects could be multiple, ranging from social, economic, psychological, lifestyle (smoking, sedentary lifestyle, improper diet) and biological (abnormal lipids, hypertension, diabetes, obesity) [2]. Genetic factors such as mutations at specific chromosomal locations and single nucleotide polymorphisms have also been implicated[3]. The INTERHEART case-control study reported that nine established risk factors (high apolipoprotein B/ A1 ratio, smoking, hypertension, diabetes, obesity, psychosocial stress, low fruit and vegetables intake, low alcohol intake and sedentary lifestyle), explained more than 90 per cent of acute myocardial infarction[4]. Prospective cohort studies in developed countries have identified that five major cardiovascular risk factors (smoking, hypertension, high LDL cholesterol, low HDL cholesterol and diabetes) are associated with CHD5. These studies also reported that more than 90 per cent of acute coronary events can be predicted by major coronary risk factors[5]. Previous case-control studies from India have reported importance of smoking, hypertension, diabetes, and abnormal lipids[6-9]. Individual studies have also studied novel risk factors such as lipid subtypes, lipoprotein (a), insulin resistance, homocysteine, and dietary factors[2]. Large studies for identification of risk factors for premature CHD among Indian subjects are not available and most are limited to 50-100 subjects[1, 2]. We hypothesized that thrombogenic risk factors (e.g., smoking, dietary antioxidant deficiency, fibrinogen, platelet functions, etc.) are important in premature CHD in Nagapattinam district **(**Indians). To test this hypothesis a casecontrol study was performed to identify association of multiple vascular risk factors, both thrombogenic and atherogenic, in subjects (<55 yr age) with an acute coronary event (myocardial infarction or unstable angina) or recent angina.

MATERIAL AND METHODS

The study protocol was approved by institutional ethics committee and a Performa was prepared for collection of data for socio-demographic characteristics such as education, occupation, income and housing status for classification of socio-economic status; previous history of risk factors such as smoking or tobacco use, hypertension, diabetes; and treatment of chronic diseases. Physical examination focused on measurement of height and weight as soon as the patient was ambulant. Blood (9-10 ml) was collected within 24 hours of admission for estimation of hematological and biochemical parameters using EDTA and plain vials respectively. Premature CHD

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was defined as first manifestation before 55 years in both men and women as per current guidelines[2]. Successive consenting patients with an acute coronary event (ST elevation or non-ST elevation myocardial infarction or unstable angina) presenting to Nagapattinam government hospital and Associated Group of Hospitals, during October 2010 to September 2011 were enrolled as cases. Those with past history of acute coronary event or those with stable angina hospitalized for routine investigations were excluded. Age- and gender-matched subjects with no clinical evidence of CHD were recruited from other hospital areas such as surgical wards, blood banks, and outpatient clinics as controls. Demographic (occupation, socio-economic status), dietary, anthropometric (body mass index, BMI), clinical (blood pressure, BP), haematological (haemoglobin, leucocyte counts, platelet counts, platelet distribution width), and biochemical [total cholesterol, high density lipoprotein (HDL) cholesterol, calculated non-HDL cholesterol, calculated low density lipoprotein (LDL) cholesterol, triglycerides, fibrinogen, homocysteine] data were collected in both groups. The investigators were trained in questionnaires and dietary assessment. Dietary data were collected using a modified validated food frequency questionnaire1[10]. This questionnaire uses recall over a 12-month period. Anthropometric measurements were performed according to WHO guidelines[11]. Haematological parameters were measured using Coulter automated cell counter. Lipids were measured using standardized biochemical methods reported earlier[12]. Fibrinogen and homocysteine were measured using ELISA techniques.

RESULTS

A total of 250 subjects who consecutively presented to the biochemistry department were enrolled, however, all clinical and biochemical details were available for 165 (152 men and 13 women) patients (acute ST elevation myocardial infarction 97, acute coronary syndromes 56, newly diagnosed stable angina 12). Age- and gender-matched controls (n=199) were also recruited. The demographic characteristics are shown in Table 2.

Table 1 Social demography status

	Cases (n=165)	Control (n=199)
Age (years)		
<40	56(33.9)	67 (33.7)
41-55	109(66.1)	132(66.3)
Sex		
1.Male	152 (92.1)	175 (89.9)
2.Female	13 (7.9)	24 (12.1)
Education		
1. Literate	43(26.2)	52(26.1)
2. <10 years education	77(46.7)	95 (47.7)
3. 10-12 years education	21(12.7)	19 (9.5)
4. Graduate	24(14.5)	33 (16.5)
Social economical status		
1.poor	29 (17.6)	31 (15.6)
2.lower middle	99 (60)	132 (66.3)
3. upper middle	33 (20)	32 (16.1)
4.upper class	4 (2.4)	4 (2)

The cases and controls were well matched for age and other socio-economic characteristics. Level of literacy and socio-economic status were also similar. Mean values of various biological variables including physical measurements, BP, and haematological and biochemical variables in cases and controls are shown in Table 2.

Table 2 Anthropometric, haematological and biochemical variables

Anthropometric	Cases n= 165	Control n=199
Age (yr)	43.3 + 5.6	43.1 + 5.9
Height (m)	1.66 ± 0.08	1.65 ± 0.08
Weight (kg)	66.53 ± 9.08	64.89 ± 8.54
	24.27 ± 3.05	23.90 ± 3.00
Body mass index (kg/m2)		
Heart rate /min	82.30 ± 12.32	81.66 ± 13.53
Systolic BP mm Hg	138.10 ± 26.26	*** 124.85 ± 21.10
Diastolic BP mm Hg	89.24 ± 15.19	81.68 ± 12.08
Haematological variables		
Haemoglobin g/dl	13.32 ± 1.78	13.78 ± 1.31
Leucocyte count 103/µm	11.93 ± 4.60	11.93 ± 5.46
Platelet count 103/µm	304.63 ± 104.39**	** 268.68 ± 432.22
Platelet volume	9.36 ± 0.94	9.47 ± 0.794
Platelet RDW	10.12 ± 0.99	10.30 ±0.73
:*Biochemical variables*:		
Total cholesterol mg/dl	218.12 ± 45.52	211.32 ± 46.08
HDL cholesterol mg/dl	37.44 ± 7.54	45.87 ± 7.74
LDL cholesterol mg/dl	146.99 ± 36.39***	129.64 ± 38.80
Triglycerides	160.97 ± 36.71	130.80 ± 41.06
VLDL cholesterol mg/dl	33.81 ± 16.57	28.90 ± 20.90
Non HDL cholesterol mg/dl	180.7 ± 45.8	165.45 ± 46.80
Fibrinogen mg/dl	280.39 ± 110.59	231.35 ± 99.55
Homocysteine ng/dl	15.36 ± 6.46	15.80 ± 4.12

Values are mean ± 1 SD; *P* *<0.05, **<0.01, ***<0.001 compared to Controls

The mean weight, height and BMI were not significantly different while systolic and diastolic BP was higher in cases (P<0.001) compared to controls. Total platelet counts were significantly more (P<0.05) in cases while platelet volume and platelet random distribution width was similar. Mean total cholesterol levels were not significantly different in cases and controls. Mean HDL cholesterol was significantly lower and non-HDL cholesterol, LDL cholesterol and triglycerides significantly greater in cases (P<0.001) than in controls. Mean fibrinogen levels were significantly (P<0.001) greater in cases and homocysteine levels not significantly different. Analysis of food intake showed that, in cases vs. controls, intake (g/day) of pulses and legumes (18.8 ± 22.5 vs. 25.1 ± 27.2), roots and tubers (60.0 ± 57.7 vs. 90.7 ± 80.9), green leafy vegetables (10.0 ± 20.1 vs. 15.1 ± 32.2), other vegetables (50.1 ± 64.4 vs. 147.0 ± 102.8) and fruits (13.9 ± 32.7 vs. 50.5 ± 56.0) was significantly lower (P<0.01) in cases. The intake (g/ day) of milk and its products (456.7 ±199.6 vs. 356.8 ± 242.2), visible fats and oils (15.7 ± 5.8 vs. 14.0 ± 5.5), ghee (13.8 ± 19.0 vs. 1.5 ± 6.7), deep fried food (15.2 ± 25.0 vs. 1.0 ± 5.1) and shallow fried foods (24.0 ± 60.4 vs. 2.7 ± 17.2) was more in cases (*P*<0.01). Intake of total calories as well as various macronutrients such as fats, saturated fats, mono- or polyunsaturated fats was significantly more (*P*<0.001) in cases while n-3 fats higher in controls. Intakes of various nutrients after adjustment for energy intake (energy%) revealed that intake of total fats, mono- and polyunsaturated fats and n-6 fats was more and intake of n-3 fats lower in cases. Intake of vitamin C, folic acid and

selenium was lower in cases. There was no difference in intake of other proximate factors or micronutrients (Table III). Presence of various risk factors was also determined in the study subjects. In cases vs. controls presence of current smoking (28.5 vs. 2.0%), low fruit and vegetables intake (88.5 vs. 79.4%), high fat intake >30en per cent (44.8 vs. 32.7%), known diabetes mellitus (15.8 vs. 9.0%), hypertension (61.8 vs. 15.4%), high LDL cholesterol >130 mg/dl (69.1 vs. 47.2%), total: HDL cholesterol >4.5 (81.2 vs. 52.8%) and triglycerides >150 mg/dl (64.2 vs. 33.2%) was significantly higher. Hyperfibrinogenaemia levels was >200 mg/dl (76.8 vs. 53.8%) as well as hyperhomocysteinaemia levels was >20 ng/ml (13.9 vs. 1.5%) were more in cases.

Table 3 Intake of various proximate and micronutrients

	Cases n= 165	**Control n=199**
Energy calorie/day	2009.81 ± 590.91	1789.12 ± 357.63
Carbohydrates g/day	297.40 ± 86.61	273.99 ± 56.54
Protein g/day	62.68 ± 17.83	59.69 ± 12.07
Fat g/day	65.22 ± 23.93	52.15 ± 15.50
Saturated fat g/day	28.99 ± 12.58***	20.77 ± 14.35
Monounsaturated fat g/day	18.88 ± 15.83	11.25 ± 7.57
Polyunsaturated fat g/day	13.97 ± 9.63	10.78 ± 4.60
n-6 fat g/day	10.56 ± 6.02	8.01 ± 3.39
n-3 fat g/day	2.35 ± 1.27	2.76 ± 1.24
Fiber g/day	7.91 + 3.57	8.15 + 2.49
Vitamin B1 mg/day	1.88 ± 0.59	1.88 ± 0.42
Vitamin B2 mg/day	1.51 ± 0.49	1.28 ± 0.42
Vitamin B3 mg/day	15.34 ± 6.77	14.93 ± 5.03
Vitamin C mg/day	39.19 ± 30.50	50.54 ± 40.06
Carotene Iu/day	1271.23 ± 1264.25***	1289.31 ± 1002.96
Folic acid mg/day	67.96 ± 25.26	75.63 ± 25.98
Iron mg/day	23.71 ± 14.02	26.49 ± 16.36

Values are given as mean ± SD. en% energy%

DISCUSSION

This study showed that thrombotic factors (smoking, low fruit and vegetables intake, high fibrinogen, high homocysteine) as well as atherogenic factors (high fat diet, hypertension, high LDL cholesterol, low HDL cholesterol and high triglycerides) were important in the development of premature CHD. Thrombotic risk factors have been considered more important in premature CHD in studies from India. Previous casecontrol studies in premature CHD from India reported smoking, hypertension and low HDL cholesterol as important risk factors1,2,4. The present study also showed the importance of both thrombogenic and atherogenic risk factors. This finding is similar to the studies on premature CHD in developed countries[5,13]. Studies among older CHD patients in India such as the INTERHEART4 and others[6-9] reported that multiple thrombogenic and atherogenic risk factors such as smoking, high apolipoprotein B, known hypertension or diabetes, high waist-hip ratio (WHR), psychosocial factors, lack of exercise and low fruit and vegetables consumption are important. The INTERHEART study also reported that younger age of onset of major risk factors explained the premature CHD in South Asians[4]. Epidemiological studies have reported younger age of onset of metabolic cardiovascular risk factors[14]. The highest risks for CHD in the present study were smoking, low fruit and vegetables intake, hypertension and low HDL cholesterol similar to studies among the premature CHD in high income countries[13]. In INTERHEART studies the adjusted ORs for these risk factors were significantly lower[15]. The age-adjusted and multivariate-adjusted ORs, for smoking, daily fruits and vegetables intake, hypertension and high ApoB/ ApoA1were substantially lower than the present study. It is likely that these risk factors are more important in younger patients. Our study also shows that emerging risk factors such as fibrinogen and homocysteine are important in young CHD patients. Prospective studies have identified fibrinogen as important cardiovascular risk factor[16]. Role of high homocysteine as cardiovascular risk factor is well established[17] and the present study shows that high levels are associated with increased risk even after multivariate adjustments. Previous case-control studies from India did not report significant association with homocysteine unlike the present study[1,2]. It may be possible that this factor is important in younger patients but larger studies are needed to confirm this finding. The statistical distribution of homocysteine level is skewed and the numbers of controls with high homocysteine are small and this is a shortcoming. We did not find significant correlation with other haematological parameters including platelet size and platelet distribution which are markers of platelet activation. The platelet counts were higher in cases than controls but this could be a marker of activation during an acute coronary event hence this was not analyzed further. Dietary factors were also studied and low fruit and vegetables intake was found to be associated with increased risk. Other associations noted were with high intake of fats, edible oils, ghee and fried foods and intake of saturated fats, mono- and polyunsaturated fat, n-6 fats and an inverse association with n-3 fats. A very low intake of ghee and shallow and deep fried foods in controls was surprising and might reflect either changing dietary habits or misreporting of fat intake. More studies are required although the dietary differences observed in the present study are similar to previous Indian studies[7,8]. There were several limitations in this study. Case control studies have large number of inherent biases especially in identification of controls. To minimize these, controls were included from the hospital as these would be from the same locations as cases. Similar strategy was used in the INTERHEART study4. Moreover, risk factors in controls were similar to population based studies in western India[12] and, therefore, controls were representative of local population. Secondly, measurements of risk factors in hospitalized patients are fraught with errors. BP values are unstable after acute coronary event, lipid levels quickly change, fibrinogen levels increase, platelet counts increase and

dietary recalls may be biased. Past history of hypertension and diabetes was asked and BP levels were measured at multiple times, lipid levels were measured within 24 h of admission but blood glucose levels were not measured for diagnosis of diabetes as performed in previous studies[4,6]. Thirdly, although the number of subjects was not large, it is the largest study of premature CHD in India. Fourthly, this was not a prospective study which could have definitely evaluated the potential impact of various risk factors. Fifthly, many emerging risk factors [lipoprotein(a), triglyceride remnants, lipid subtypes, insulin resistance, C-reactive protein, inflammatory factors] or genetic markers that have been implicated in premature CHD, were not studied[2,3]. And, finally, as the number of women in this study was small, the risk factors could not be generalized. The strengths of the study include a robust case-control design, large numbers, enrollment of fresh cases, and strict biochemical and statistical criteria for analyses.

CONCLUSION

In conclusion, this study shows that premature coronary artery disease in Indians is due to combination of thrombosis and atherosclerotic vascular risk factors. These risk factors are highly prevalent in the community1. Prevention and control of premature cardiovascular diseases in India needs urgent control of these factors. Improving lifestyles with tobacco cessation, diet modulation with more fruits and vegetables and lower fat intake, and increased physical activity are critical. Target oriented control of hypertension, lipid levels and glycaemia is required. The study adds that lowering fibrinogen and homocysteine using novel strategies as well as control of all the risk factors from an early age are essential to prevent premature disease.

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