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Impact of Epidemiological and Clinical Risk factors in the Pathogenesis of Coronary Heart Disease

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Abstract

Coronary heart disease (CHD) is a multifactorial disease resulting due to accumulation of atheromatous plaques within the walls of the coronary arteries that supply the myocardium (the muscle of the heart) with oxygen and nutrients. The aim of the present study is to investigate association of epidemiological and clinical risk factors in the development of disease in CHD patients and their first degree relatives (FDRS) in comparison with controls. The aetiology of CHD is multifactorial. It is the result of interaction between genetic, lifestyle and environmental factors. Monitoring the epidemiology and clinical risk factors of CHD is an important component in the pathogenesis of CHD.

Introduction

Coronary heart disease (CHD) is a multifactorial disease resulting due to accumulation of atheromatous plaques within the walls of the coronary arteries that supply the myocardium (the muscle of the heart) with oxygen and nutrients. CHD is also called as a coronary artery disease (CAD) or coronary atherosclerosis is the major cause of morbidity and mortality in the developed world. The magnitude of this problem is profound, as CHD claims more lives than all types of cancer combined and the economic costs are also considerable. Although currently a problem of the developed world, the World Health Organization predicts that global economic prosperity could lead to an epidemic of atherosclerosis as developing countries acquire Western habits (Sawant et al., 2011).

The Indian Asian population accounts for a fifth of all global deaths from coronary heart disease (CHD). CHD deaths on the Indian subcontinent have doubled since 1990, and are predicted to rise a further 50% by 2030 (Tan et al., 2014).

Patients with a greater number of measurable CHD risk factors may have atherosclerotic progression of disease at a much faster rate (younger age) compared with those with few or no risk factors, such as conventional (age, gender), Non modifiable (Diabetes Mellitus, Family History) and Modifiable risk factors (Obesity, Hypertension, Smoking, Food Habits, Physical activity and Dyslipidemia) (Canto et al., 2011).

The oxidative modification hypothesis of atherosclerosis states that circulating LDL particles are modified by oxidation and that these modified particles are then taken up by macrophages inside the arterial wall. Such cholesterol-laden macrophages form the start of atherosclerotic plaques and initiate the events that culminate in the formation of a fibrous plaque. Rupture of fibrous plaque leads to thrombus formation and occlusion of the vessel (Zock and Katan, 1998; Nageswara et al., 2005).

The oxidative degradation of lipids is referred as a Lipid peroxidation, it is the process in which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. Oxidized low density lipoproteins (oxiLDLs) may also exert several proinflammatory effects that can contribute to the development of CHD (Cavalca et al., 2001).

Elevated ROS ensures cell proliferation, hypertrophy, growth arrest, apoptosis and oxidation of lipids, proteins and DNA. DNA damage ranges from 'macro' damages (including microdeletions, insertions in chromosomes) to 'micro' damages (induces oxidative DNA damage which includes strand breaks, base and nucleotide modifications, particularly in sequences with high guanosine content). Oxidative modification induces a robust repair response, characterized by excision of modified bases and nucleotides and further double-stranded DNA breaks also activate DNA repair enzymes. There is increasing evidence to suggest that DNA damage to cells within the atheromas plays an important role in both atherogenesis and the behaviour of established lesions (Martin, 2001).

Nitric Oxide (NO) is a most important free radical produced by endothelial cells and plays a vital role in vascular relaxation, displays vasoprotective effect by scavenging superoxide radicals and suppressing platelet aggregation, leukocyte adhesion and smooth muscle cell proliferation and signaling (De, 2000; Marsden et al., 1993).

NO also plays a role in oxidative damage of the DNA. Reaction of NO with O_2- leads to oxidative DNA damage due to the formation of peroxynitrite which may have OH- like oxidizing potential. NO is actually a vasodialator, synthesized from L-arginine by a group of enzymes called nitric oxide synthases. Any change in the genetic constitution of the genes encoding NOS enzymes, leads to the impaired function of NO which in turn leads to the development of CHD (Cook, 2006; Soydinc et al., 2007).

There is a strong association between systemic inflammation and coronary artery disease; this association is thought to be causal i.e. inflammation increases the risk of the disease, rather than simply marking the presence of atherosclerosis which is itself an inflammatory process (Woods et al., 2000) and circulating factors related to inflammation may be predictors of cardiovascular disease in general populations (Kuller et al., 1996). Progressive inflammation leads to activation of inflammatory cytokines, recruitment of inflammatory cells, generation of free radical species and subsequent malignant transformation.

Earlier studies have shown that C-reactive protein (CRP) may have direct proinflammatory effects, and contribute to the initiation, and progression of atherosclerotic lesions (Koenig et al., 2007).

As compared to other ethnic groups, prevalence of CHD in the younger groups is much higher in Indians and they are at extreme prematurity of CHD where the rates are 2 to 4 fold higher overall and 5 to 10 fold higher in those younger than 40 years of age, irrespective of gender, region, or social class. Manifestation of CHD in a more extensive form at a younger age despite a relatively low longitudinal burden of conventional risk factor points to some other risk factors, presumably genetically determined, which predispose our population to an increased risk at a much younger age (Kasliwal et al., 2006).

Objective

The aim of the present study is to investigate association of epidemiological and clinical risk factors in the development of disease in CHD patients and their first degree relatives (FDRS) in comparison with controls.

Materials and Methods

The study population consisted of 300 patients with angiographically documented CAD, admitted at the cardiology unit of Durgabai Deshmukh Hospital and Research Center, Hyderabad and 100 asymptomatic FDRS of the patients and 300 healthy individuals with no known history of any disease, as controls. The healthy controls subjects representing same geographical location without any history of CAD were included after a thorough interview and clinical examination. Patients with concomitant valvular heart disease, cardiomayopathy, acute renal failure, acute and chronic viral or bacterial infections, asthama, tumours or connective tissue diseases and those who are on dialysis were excluded from the study.

This study was approved by Institutional Ethics Committee for Biomedical Research of Institute of Genetics and Hospital for Genetic Diseases, Hyderabad.

Detailed written informed consent was obtained from all the participants of this study. All the subjects were examined clinically and detailed history was recorded with particular reference to the known risk factors for CAD, including family history, hypertension, diabetes mellitus, smoking, food habits, life style etc.

Following an overnight fast, blood samples were drawn by vein puncture into two tubes, with and without anticoagulant for biochemical and molecular analysis. Estimation of lipid profiles, Hs-CRP and genotypic evaluation of IL-18 polymorphism was carried out in the samples of CAD, FDRs and controls.

Evaluation of Epidemiological risk factors by statistical analysis, Clinical Risk factors were estimated by Commercially available kits for lipid profiles, CRP from Randox Laboratories, United Kingdom and conventional methodologies for Lipidperoxidation by Gavino et al 1981, Nitric Oxide (Nitrite / Nitrate) by Lepoivre et al 1990, Comet assay by Singh et al 1988, Total Antioxidant Status (TAS) by re et al 1999, Total Oxidant Status (TOS) by Ozcan Erel 2005 and OSI by formula (OSI=(TOS.

Statistical analysis

Statistical analysis for our datawas performed using version 9, (SAS Institute Inc, Cary, NC, USA). Continuous clinical data was compared by unpaired Student's t-test and presented as mean ± standard deviation (SD). The $\chi 2$ test was used to compare discrete variables. A p value of <0.05 was considered to be statistically significant. A two-tailed probability test of 0.05 or less was considered statistically significant.

Results

The number of CHD patients, FDRS and controls along with their demographic data is presented in Table 1(categorized). The risk factors for coronary heart disease include gender, history of hypertension and DM, family history, smoking (male). The ratio between male to females in CHD patient was about 3.2:1, FDRS 2.3:1 and controls 1.2:1 respectively.

	Controls	*CHD patients*	*Chi Square*			*FDRS*	*Chi Square*		
	N (%)	*N (%)*	*Pearson value*	*Df*	*Fisher Exact Test*	*N (%)*	*Pearson vale*	*Df*	*Fisher Exact Test*
Male	168 (56)	229 (76.3)	27.70	1	0.001*	70 (70)	6.10	1	0.01*
Female	132 (44)	71 (23.7)				30 (30)			
Veg	82 (27.3)	86 (28.7)	0.132	1	0.785	26 (26)	0.068	1	0.89
Non veg	218 (72.7)	214 (71.3)				74 (74)			
Smoker	105 (35)	127 (42.3)	3.401	1	0.07*	30 (30)	0.839	1	0.39
Non Smoker	195 (65)	173 (57.7)				70 (70)			
DM	0	87 (29)	101.7	1	0.001*	13 (13)	40.31	1	0.001*
No DM	300	213 (71)				87 (87)			

With Family History	0	20 (6.7)	20.690	1	0.001*	100 (100)	363.5	1	0.001*
Without Family History	300	280 (93.3)				NIL			
With HTN	0	116 (38.7)	143.8	1	0.001*	51 (51)	175.35	1	0.001*
Without HTN	300	184 (61.3)				49 (49)			
*Significant at p<0.05									

Table 1: Demographic data of Controls, FDRS and CHD patients (Categorized).

The Continuous demographic data is presented in Table 2. The risk factors for coronary heart disease include age, Blood Pressure, were significantly associated with CHD patients followed by FDRS compared to controls while BMI found to be normal between three groups.

	Controls N=300					***FDRS N=100***					***CHD N=300***				
	Mean ± SD	***Mean Error***	***Range***	***Min***	***Max***	***Mean ± SD***	***Mean Error***	***Range***	***Min***	***Max***	***Mean ± SD***	***Mean Error***	***Range***	***Min***	***Max***
Age (Years)	52.01± 8.08	0.46	50	23	65	42.60± 15.3*	1.5	67	18	58	54.26± 12.06*	0.697	64	26	65
BMI (Kgm^2)	25.35± 5.1	0.29	25	17	42	25.41± 4.9	0.49	24	16	40	28.66± 4.797	0.277	27	15	42
Sy-BP (mmHg)	121.65± 7.2	0.419	33	98	131	124.4± 14.3	1.43	90	90	180	135.73± 21.8	1.260	120	80	200
Di-BP (mmHg)	80.97± 5.4	0.312	30	60	90	83.20± 9.8	0.98	50	60	110	90.29± 11.8	0.685	70	50	120
***Significant at p<0.05**															

Table 2: Demographic data of Controls, FDRS and CHD patients (Continuous).

Total cholesterol, Triglycerides, LDL levels were significantly high (p<0.05) in CHD patients and FDRS compared to the control group while, HDL cholesterol levels were low in patients followed by FDRS compared to controls (p<0 .05) as given in Table 3.

	Controls N=300					***FDRS N=100***					***CHD N=300***				
	Mean ± SD	***Mean Error***	***Range***	***Min***	***Max***	***Mean ± SD***	***Mean Error***	***Range***	***Min***	***Max***	***Mean ± SD***	***Mean Error***	***Range***	***Min***	***Max***
CHO (mg/dL)	166.47± 32.71	1.889	170	96	203.1	176.60± 39.7*	3.97	233	65	231.3	247.91± 39.8*	2.299	187	114	301
TRI (mg/dL)	152.46± 26.67	1.54	105	93	158	147.6± 44.36	4.4	234	53	287	174.62± 37.7*	2.178	236	20	256
HDL (mg/dL)	44.72± 11.047	0.638	48	20	52	35.5± 12.12*	1.21	53	16	38	33.51± 11.51*	0.665	59	9	68
LDL (mg/dL)	90.90± 33.29	1.922	155	31	186	125± 50.3*	5.03	234	56	236	179.48± 36.4*	2.240	233	96	260
VLDL (mg/dL)	30.42± 5.2	0.305	21	19	40	29.02± 9.3	0.93	51	6	57	30.62± 12.37	0.714	74	6	80
*Significant at p<0.05															

Table 3: Lipid profiles of Controls, FDRS and CHD patients.

	Controls N=300					FDRS N=100					CHD N=300				
	Mean ± SD	Mean Error	Range	Min	Max	Mean ± SD	Mean Error	Range	Min	Max	Mean ± SD	Mean Error	Range	Min	Max
MDA (n moles/ml)	2.10± 1.43	0.083	10	1	11	5.18± 2.7*	0.27	11	1	12	7.07± 2.5*	0.146	10	2	12
NO (µmoles/ml)	1.88± 0.89	0.052	7	0	7	3.44± 1.16*	0.11	5	1	6	4.02± 1.1*	0.066	7	1	8
CRP (mg/L)	10.82± 9.6	0.557	42	6	48	41.3± 49.01*	4.9	186	6	192	73.24± 63.1*	3.644	186	6	192
COMET (µm)	10.59± 1.6	0.098	9	5	14	16.78± 6.6*	0.66	27	8	35	22.18± 5.4*	0.316	49	8	57
TAS (µmol Trolox Eq/L)	819.20± 76.21	4.400	420	564	984	663.7± 107.8*	10.7	464	459	923	548.25± 54.1*	3.125	272	402	674
TOS (µmol H_2O_2 equiv./L)	12.95± 1.798	0.104	7	9	16	17.1± 6.5*	0.65	21	9	30	24.13± 3.2*	0.187	12	18	30
OSI (arbitrary unit)	1.59± 0.493	0.028	1	1	2	2.88± 1.4*	0.14	6	1	7	4.53± 0.79*	0.046	4	3	7
*Significant at p<0.05															

Plasma MDA, Nitric Oxide (NO) or (Nitrite/Nitrate), TAS, TOS, OSI and CRP levels were found to be high in the CHD patients, FDRS compared to the controls at p<0.05. Comet tail length (oxidative DNA damage) of patients and FDRS was found to be significantly high at p<0.05 compared to controls as summarized in Table 4.

Table 4: Oxidative Stress and Inflammatory Markers in Controls, FDRS and CHD patients.

Diastolic Blood pressure, Total cholesterol, triglycerides, LDL cholesterol, VLDL, NO, Comet tail length, TAS, OSI and CRP were shown significant Correlation with CHD patients as summarized in Table 5.

		AGE	BMI	Sys BP	Dia BP	CHO	TRI	HDL	LDL	VLDL	MDA	NO	CRP	COMET	TAS	TOS	OSI
AGE	r	1															
BMI	r	-0.11	1														
Sys BP	r	0.001	0.029	1													
Dia BP	r	-0.05	0.072	.648**	1												
CHO	r	*.124**	*-.126**	-0.01	0.029	1											
TRI	r	0.066	*-.143**	0.055	0.031	.493**	1										
HDL	r	-0.02	-0.023	-0.044	-0.072	-.16**	-.50**	1									
LDL	r	0.048	0.019	-0.016	0.038	.481**	-.22**	0.009	1								
VLDL	r	0.096	*-.146**	0.043	0.005	.478**	.933**	-.44**	-.2**	1							
MDA	r	-0.04	0	-0.012	-0.027	0.066	0.095	-0.035	-0.06	0.098	1						
NO	r	0.079	0.028	-0.056	*-.127**	0.041	0.017	0.004	0.087	0.022	.168**	1					
CRP	r	-0.06	*-.114**	-0.068	-0.021	.188**	*.137**	-0.045	-0.00	.157**	.205**	-0.071	1				
COMET	r	-0.08	-0.014	-0.039	-0.061	-0.061	0.013	0.098	-0.10	0.025	.154**	.184**	0.048	1			
TAS	r	0.03	0.081	0.092	0.015	0.025	-0.028	0.075	*.130**	-0.049	-0.095	-0.064	-0.05	-0.027	1		
TOS	r	-0.07	0.056	0.042	0.096	*-.115**	-0.007	0.025	*-.13**	-0.022	0.099	-0.036	0.112	-0.061	0.08	1	
OSI	r	-0.06	0.042	-0.04	0.038	*-.119**	-0.014	0.008	-.1**	-0.018	*.121**	-0.013	0.071	-0.019	-.55**	.68**	1
	N	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300
N= No of Samples. r = Pearson's correlation coefficient. **Correlation is significant at the 0.05 level (2-tailed)* **Correlation is significant at the 0.01 level (2-tailed)																	

Table 5: Pearson's correlation coefficient for the correlation between Clinical variables in CHD patients.

Diastolic Blood pressure, triglycerides, LDL cholesterol, VLDL, MDA, NO, Comet tail length, TOS, OSI and CRP were shown significant Correlation with FDRS as summarized in Table 6.

	AGE	*BMI*	*Sys BP*	*Dia BP*	*CHO*	*TRI*	*HDL*	*LDL*	*VLDL*	*MDA*	*NO*	*CRP*	*COMET*	*TAS*	*TOS*	*OSI*	
AGE	r	1															
BMI	r	0.131	1														
Sys BP	r	0.161	0.036	1													
Dia BP	r	-0.06	-0.052	.52**	1												
CHO	r	.250*	0.035	.20*	0.044	1											
TRI	r	0.004	-0.111	-0.037	0.052	.28**	1										
HDL	r	0.003	0.041	0.081	0.067	-0.074	-.31**	1									
LDL	r	.34**	0.195	.35**	0.075	.71**	-.221*	0.19	1								
VLDL	r	-0.06	-0.051	-0.047	0.057	.27**	.91**	-.234*	-.25*	1							
MDA	r	.34**	.216*	0.123	-0.06	.36**	-0.185	0.105	.59**	-0.195	1						
NO	r	0.159	.27**	0.192	-0.07	0.094	-0.146	0.025	.31**	-0.161	.46**	1					
CRP	r	.26**	0.086	0.018	-0.12	.43**	-0.019	0.107	.37**	0.001	.39**	0.155	1				
COMET	r	.217*	0.179	0.187	-0.01	.226*	-.26**	.26**	.52**	-.31**	.64**	.52**	.248*	1			
TAS	r	-.3**	-.256*	-.253*	0.028	-.227*	0.004	-0.079	-.42**	0.076	-.60**	-.49**	-.3**	-.62**	1		
TOS	r	.49**	.30**	.38**	-0.04	.37**	-0.055	0.068	.62**	-0.157	.66**	.56**	.37**	.66**	-.7**	1	
OSI	r	.46**	.29**	.31**	-0.06	.31**	-0.046	0.064	.56**	-0.15	.65**	.57**	.34**	.68**	-.8**	.94**	1
	N	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

N= No of Samples. r = Pearson's correlation coefficient. **Correlation is significant at the 0.05 level (2-tailed)* **Correlation is significant at the 0.01 level (2-tailed)

Table 6: Pearson's correlation coefficient for the correlation between Clinical variables in FDRS.

Regression analysis has been performed against HDL vs Total cholesterol, triglycerides, LDL cholesterol, VLDL, MDA and NO in CHD patients and FDRS and found that cholesterol, triglycerides, LDL and VLDL were significantly associated in decrease of HDL levels in CHD Patients and FDRS.

Dependent variable		*Predictor variables*	*Un standardized Coefficients*		*t*	*P value*
			B	*SE*		
CHD Patients	HDL	(Constant)	51.199	3.998	12.805	0.0001
		CHO	0.111	0.023	4.897	.0001*
		TRI	-.172	0.027	-6.306	.0001*
		LDL	-.108	0.021	-5.053	.0001*
		VLDL	0.084	0.125	0.668	0.504
		MDA	-.073	0.223	-.325	0.745
		NO	0.349	0.492	0.710	0.478
FDRS	HDL	(Constant)	49.635	6.655	7.458	0.0001
		CHO	-.131	0.056	-2.348	0.021*
		TRI	-.163	0.064	-2.540	0.013*
		LDL	0.131	0.047	2.759	0.007*
		VLDL	0.709	0.317	2.238	0.028*
		MDA	-.074	0.547	-.135	0.893
		NO	-1.018	1.117	-.912	0.364

*Significant at p<0.05

Table 7: Regression analysis: relationship between the Lipid profiles, Oxidative Stress Variables vs HDL among CHD patients and FDRS.

Regression analysis has been performed against Lipid profiles and Oxidative Stress Variables vs COMET in CHD patients and FDRS and found that MDA and NO were significantly associated with comet tail length in CHD Patients and FDRS.

Dependent variable		*Predictor variables*	*Un standardized Coefficients*		*t*	*P value*
			B	*SE*		
CHD Patients	COMET	(Constant)	20.362	2.249	9.053	0.0001
		CHO	-.003	0.013	-.265	0.791
		TRI	-.007	0.015	-.436	0.663
		LDL	-.014	0.012	-1.179	0.239
		VLDL	0.028	0.070	0.398	0.691
		MDA	0.262	0.126	2.083	0.038*
		NO	0.830	0.277	3.003	0.003*
FDRS	COMET	(Constant)	7.274	2.786	2.611	0.011
		CHO	-.014	0.023	-.590	0.557
		TRI	0.022	0.027	.825	0.411
		LDL	0.030	0.020	1.528	0.130
		VLDL	-.177	0.133	-1.331	0.186
		MDA	0.977	0.229	4.263	0.0001*
		NO	1.432	0.468	3.062	0.003*
*Significant at p<0.05						

Table 8: Regression analysis: relationship between the Lipid profiles, Oxidative Stress Variables vs COMET among CHD patients and FDRS.

Regression analysis has been performed against oxidative stress variables vs TAS in CHD patients and FDRS and found that TOS and OSI were significantly associated with decrease of TAS levels in CHD Patients and FDRS.

Dependent variable		*Predictor variables*	*Un standardized Coefficients*		*t*	*P value*
			B	*SE*		
CHD Patients	TAS	(Constant)	557.545	16.972	32.852	0.0001
		LDL	0.033	0.045	0.733	0.464
		MDA	-.734	0.672	-1.092	0.276
		NO	-2.098	1.477	-1.421	0.157
		TOS	14.739	.703	20.962	.0001*
		OSI	-78.898	2.915	-27.063	.0001*
FDRS	TAS	(Constant)	790.705	20.153	39.235	0.000
		LDL	0.015	0.140	0.104	0.917
		MDA	-6.898	2.689	-2.565	0.012
		NO	-2.525	5.481	-.461	0.646
		TOS	14.008	2.595	5.399	0.0001*
		OSI	-112.750	11.087	-10.170	0.0001*
*Significant at p<0.05						

Table 9: Regression analysis: relationship between the Oxidative Stress Variables vs TAS among CHD patients and FDRS.

Discussion

Epidemiological Risk Factors

Gender distribution

Traditionally, CHD has been considered a disease of men. However, CHD is the leading cause of death both in men and women (Beltrame et al., 2012; Mikhail, 2005). Although the initial manifestation of CHD is delayed in females by about ten years compared to males, there is an abrupt increase in CHD mortality rates for females immediately following menopause but a progressive increase over subsequent years (Beltrame et al., 2012). Women are twice as likely to die of a first myocardial infarction (MI) (Banks, 2008), and have a less favorable long-term survival as compared with men. As women have smaller coronary vessels than men, they are also twice as likely to die as a result from coronary artery bypass surgery, and are more likely to undergo repeat revascularization (Eastwood and Doering, 2005).

In the present study, we have also found significant elevation in number of male cases compared to females in CHD patients (76.3%, 23.7%) as shown in table 1. A global case-control study has also revealed the males are more prone to CHD at younger age than females (Anand et al., 2008). However, the differences in lifestyle factors do not fully explain the differences in CVD incidence between the genders. Genetic factors also contribute to CHD and stroke susceptibility (Lusis et al., 2004).

Age at diagnosis

Ageing is an un-modifiable risk factor for CHD (Lerner and Kannel, 1986). The WHO reports that the principal cause of death of people over 65 years is CHD. The ageing population of many countries has accelerated the contribution of CHD to total disease burden as a result of long-term exposure to other risk factors. It is predicted that the global ageing population will maintain CHD as a predominant cause of death worldwide (Mensah, 2004).

In contrast to these developed countries, South Asian countries (such as India, Pakistan, Bangladesh, Sri Lanka, and Nepal) the highest prevalence of myocardial infarction is seen in those younger than 40 years of age, whereas it is less marked in those older than 60 years (Joshi et al., 2007).

In accordance with above studies we have also found the mean age (in years) of CHD patients to be 54.26±12.06 and that of the controls to be 52.01±8.08 and further we have included FDR's with mean age 42.60±15.3 (Table 2).

The prematurity age of onset <55 years for men and >65 years for women in first-degree relatives (NCEP, 2002). If family history characteristics beyond premature CHD in first-degree relatives also increase CHD risk, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) guidelines might underestimate CHD risk and the need for lipid-lowering therapies or other preventive interventions for individuals with such histories (NCEP, 2002).

Diabetes mellitus (DM)

Diabetes mellitus (DM) is a multifactorial disorder as is CHD. Neither of them is simple in its genesis but appears to be the outcome of a constant interaction between nature and nurture (Das and Tripathy, 1995). The prevalence of CHD is 2 to 4 times more in DM patients compared to non-DM.

In the present study we have found 29% of CHD patients with DM when compared to controls indicating the importance of DM as a risk factor for development of CHD (Table 1). Similarly Mohan et al., 2007 suggested DM as a risk factor for CHD in south Indian population. The development of CHD in DM is dependent on underlying genetic predisposition and coexisting independent accelerating factors such as hypertension and Dyslipidemia (Pop-Busui et al., 2014).

Family history

Family history is a simple yet powerful clinical tool. Family members resemble each other in risk of disease because of shared biological, cultural, and behavioral factors. Biologically, first-degree relatives (dizygotic twins, full siblings, and parent-child) share 50% of their genes, second-degree relatives (half-sibling, aunt/uncle/niece/nephew, and grandparent-grandchild) share 25%, and third- degree relatives (first cousins) share 12.5%. In terms of culture and behavior, close relatives often share environments for a sizable portion of their lifetimes and may have long-lasting lifestyle and nutritional habits and cultural attitudes in common (Valdez et al., 2010).

Although traditionally associated with the evaluation of rare Mendelian single-gene disorders in individuals and their relatives, family history can also play an important role in risk assessment and prevention of common chronic diseases. Numerous studies on diseases of major public health importance (e.g., cancer, heart disease, diabetes, and stroke) consistently show that the odds of developing one of these conditions are significantly increased by having one or more close relatives with the disease (Flossmann and Rothwell, 2005).

Recent epidemiological studies have reported that a family history of CHD is an autonomous risk factor for CHD development. In particular, having a family history is associated with 1.5 to 2 fold increase in risk of developing cardiovascular disease. Furthermore, maternal family history of CHD may even predict future cardiovascular events more strongly than a paternal family history.

Results of the present study revealed 6.7% of CHD patients with family history of CHD as shown in table 1 and we have also included 100 FDRS of CHD patients to identify the at risk individuals.

Prevalence of CHD in the young is much higher in Indians when compared to other ethnic group and first-degree relatives (FDR's) of coronary artery disease patients are at risk of 12.1 folds, correlating with earlier age-of-onset and poor prognosis (Svati et al., 2009).

Body Mass Index (BMI)

Obesity is recognized as an important risk factor for various diseases. Studies have indicated an increase in all cause mortality with increased body mass index (BMI), especially death from cardiovascular disease in men. The risk of disease appears to increase as a function of the percent fat content in the body, above an upper limit of normal (Singh et al., 2008). Accepted BMI value of >25 kg/m^2 and 23 kg/m^2 as the cut-off for obesity for Asian men and women respectively (Singh et al., 2008).

Multiple epidemiological studies have demonstrated increased morbidity and mortality with BMI 30 kg/m^2. Data from the Prospective Studies Collaboration, which analyzed 900000 adults, demonstrated a 30% increase in all-cause mortality for every increase of 5 U in BMI above a BMI of 25 kg/m^2 (Andre Cornier et al., 2011). However, in the present study BMI is found to be normal between 3 study groups shown in Table 2.

Hypertension/BP

Hypertension is a major independent risk factor for the development of CHD, stroke, and renal failure. Epidemiological studies have established a strong association between hypertension and CHD. An evaluation and Treatment of High Blood Pressure recommendation has defined "hypertension" as a BP of 140/90 mm Hg. There is a strong but complex association of BP and age. Until about 50 years of age, systemic blood pressure (SBP) and diastolic blood pressure (DBP) rise in tandem. After 50 years of age, SBP continues to rise steadily, whereas DBP tends to fall. The prevalence of systolic hypertension is thus directly proportional to the age of the population (Rosendorff et al., 2007).

In the present study history of hypertension was shown in 38.7% of CHD patients and 51% of FDRS as shown in Table 1. A study by Gupta, 2004 reported that HT is directly responsible for 24% of all CHD deaths in India. Various factors have been attributed to this rising trend of HT which include urbanization such as change in life style pattern, diet, stress etc. (Das et al., 2005).

Smoking

Smoking is one of the main cause of CHD worldwide and epidemiologic studies strongly support the assertion that smoking in both men and women increases the incidence of MI and fatal CHD (Ambrose and Barua, 2004).

In the present study the habit of smoking was found in 42.3% of male CHD patients, 30% FDRS and 35% controls (Table 1). Begom and Singh, 1995 also reported that the prevalence of smoking in south Indian males was significantly high (44.6%) compared to north Indians (36.9%). Guptha, 2008 reported smoking as an independent risk factor for MI in Indian population.

Smoking is a contributory factor for CHD as it alters endothelial function, the redox state, inflammation, and global DNA methylation, which is associated with one-carbon metabolism and the transsulfuration pathway (Campesi et al., 2013).

Food Habits

In the present study we have found that 71.3% of non vegetarians in CHD patients, 74% in FDRS and 72.2% in controls as shown in table 1.

Many epidemiological studies have indicated a protective role for a diet rich in fruits and vegetables against the development and progression of cardiovascular disease (CVD), Physical inactivity and unhealthy eating contribute to CHD. Some prospective studies showed a direct inverse association between fruit and vegetable intake and the development of CVD incidents such as acute plaque rupture causing unstable angina or myocardial infarction and stroke. Many nutrients and phytochemicals in fruits and vegetables, including fiber, potassium, and folate, could be independently or jointly responsible for the apparent reduction in CVD risk (Louis et al., 2007).

Clinical Studies

Cholesterol

Atherosclerosis manifests clinically in middle and late adulthood, but it is known to have a long asymptomatic phase of development that begins early in life, often during childhood, and is significantly related to Dyslipidemia. Dyslipidemia, characterized by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C) and triglyceride (TG) levels as well as low high-density lipoprotein cholesterol (HDL-C) concentration, is well-known risk factor for cardiovascular disease (CVD) (Fen Zhu et al., 2012).

As high total cholesterol levels are considered to be a major independent risk factor for development of CHD, considerable attention has been directed towards evaluating the impact and mechanism of cholesterol lowering therapies and interventions for cardio- vascular outcomes (Lloyd-Jones et al., 2009). Cholesterol has been shown to interrupt and alter vascular structure and functions as it builds within the lining of the vascular wall, and can interfere with endothelial function leading to lesions, plaques, occlusion, and emboli.

With specific relevance to the microcirculation, it has been clearly demonstrated that evolution of hypercholesterolemia is associated with endothelial cell dysfunction. Additionally, reports have shown a near-complete abrogation in vascular nitric oxide (NO) bioavailability, elevated oxidant stress, and the creation of a strongly pro-inflammatory condition; symptoms which can culminate in profound impairments to vascular reactivity (Stapleton et al., 2010).

In the present study higher levels of Cholesterol were found in CHD patients when compared to controls (Table 3) in concordance with Khaki-khatibi et al (2012), Arsenault et al (2010) and we have further found Cholesterol levels in FDRS to be lower than the patients but higher than that of the controls.

As cholesterol accumulate in arteries and initiates pathogenesis of CHD and identification of such abnormal levels may help in targeting asymptomatic individuals who are at risk for cardiovascular disease.

Triglyceride

Many epidemiological studies have reported associations between triglyceride (TRI) concentrations and the risk of CHD, but their relevance to disease remains uncertain. HDL-C usually eliminates or substantially diminishes the role of TG as a predictor of CHD. The observational studies on role of TRI have been confirmed by the models of macrophage and endothelial cells, that lipoprotein particles increase sterol delivery or reduce sterol efflux or that promote an inflammatory response, increase the expression of coagulation factors or leukocyte adhesion molecules, or impair responses that produce vasodilation are considered atherogenic (Miller et al., 2011).

In the present study higher levels TG was found in CHD patients followed by FDRS compared to controls as shown in table 3. Goldberg et al (2011), Miller et al (2011) investigated the relationship between TG levels and found that triglyceride level as an independent CHD risk factor.

High Density Lipoprotein (HDL)

Several studies have demonstrated an inverse correlation between plasma high-density lipoprotein (HDL) and the risk of CHD, and have concluded that HDL as a powerful predictor of the disease (Savel et al., 2012; and Barter et al., 2007). Reverse cholesterol transport is a multistep process that results in the net movement of cholesterol from peripheral tissues back to the liver via the plasma compartment. There is evidence that the protective effect of HDL is not achieved solely by its role in reverse cholesterol transport, but also by several other mechanisms, including its antioxidative and anti inflammatory activities (Cockerill and Reed, 1999).

In the present study we have found low levels of HDL in CHD patients and FDRS compared to controls (Table 3). Savel et al (2012), Khaki-khatibi et al (2012) and Saku et al (1999) have also found that the low levels of HDL is significantly associated with CHD.

Low Density Lipoprotein (LDL)

An increased low density lipoprotein (LDL) cholesterol concentration is a well-known risk factor for the development and progression of CHD (Schamberger et al., 2000). The process of LDL modification involved in the phenotypic change of macrophage to foam cells in the evolving stages of atherosclerosis, has established that oxidative modification of LDL is an important atherogenic factor. Much of the recent interest in oxidized LDL comes from the discovery that it exihibits properties in vitro that could explain the migration of monocyte macrophages in to the intimal space and their conversion in to foam cells (Ghosh et al., 2006).

In the present study we have observed higher levels of LDL in CHD patients followed by FDRS compared to controls (Table 3). Studies by Gawron-Skarbek et al (2014), Arsenault et al (2010) and Ghosh et al (2006) have demonstrated that the elevated levels of LDL are strongly associated with CHD.

Very Low Density Lipoprotein (VLDL)

Increased levels of VLDL-C are thought to reflect the presence of particles called lipoprotein remnants that are intermediate particles on the pathway of conversion of VLDL to LDL. When high levels of VLDL are present, the conversion of VLDL to LDL is slowed and the accumulation of intermediate particles is thought to contribute to the development of CHD.

Recently, more complex lipid analysis has shown that lipolysis of VLDL leads to release of a number of additional potentially toxic oxidized fatty acids. Studies have proved that the VLDL is promotes inflammation by macrophage cytotoxicity, expression of adhesion molecules and promotion of coagulation (Goldberg et al., 2011).

In the present study we found elevated levels of VLDL in CHD patients followed by FDRS compared to controls as shown in table 3. Ghosh et al (2006), Real et al (2001) demonstrated that the VLDL is one of the risk factor for the pathogenesis of CHD.

Malondialdehyde (MDA)

Oxidative stress-induced modifications have been implicated in many disease pathways. One disease widely purported to be associated with elevated oxidative stress is atherosclerotic CHD. Mammalian cells have a complex network of antioxidant system to scavenge

reactive oxygen species. Oxidative stress ensues when ROS evade or overwhelm antioxidants i.e oxidative stress occurs as a consequence of an imbalance between reactants, such as reactive oxygen species (ROS) and antioxidants (Afanas'ev, 2011).

Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules like DNA, proteins, lipoproteins including lipid membranes. Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction. Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when lipid peroxidation increases. The effects of lipid peroxides i.e. endothelial cell damage, uncontrolled lipid uptake, decreased prostaglandin synthesis and associated thrombogenecity are strongly implicated in the pathogenesis of atherosclerosis (Kaur et al., 2008).

The results of the present study revealed that the mean values of MDA are high in CHD patients followed by FDRS and were significant at $p<0.05$ (Table 4). Similarly, studies by Khaki-khatibi et al. (2012); Kaur et al. (2008) have also observed high levels of MDA in atherosclerotic patients. Thus oxidative stress is believed to play a significant role in the initiation and progression of atherosclerosis.

Nitric Oxide (NO)

NO is a crucial mediator of endothelium-dependent vasodilation and plays a role in platelet aggregation and in maintaining the balance between smooth muscle cell growth and differentiation. Under pathophysiological conditions, ROS and NO react to generate toxic reactive nitrogen species, particularly dinitrogen trioxide and peroxynitrite ($ONOO^-$) and cause significant damage to cellular components (proteins, membranes, nucleic acid), leading to chromosomal alterations, protein nitration, lipid peroxidation, subsequent cellular dysfunction and cellular death (Omer et al., 2012; Cooke, 2003).

Soydinc et al (2007) observed high levels of NO in CHD patients compared to controls. Similarly we have observed high levels of nitrite/nitrate in CHD patients followed by FDR's compared to healthy controls (Table 4). Growing evidence has demonstrated the importance of nitric oxide (NO) and its role in the pathogenesis of CHD (Cook, 2006).

DNA damage by comet assay

Excess ROS may induce the formation of oxidative DNA damage, DNA strand breaks, base modifications and chromosomal aberrations. Available evidence has shown that DNA damage if not repaired, may lead to deteriorated gene expression, development of a number of diseases such as cancer, diabetes, neurodegenerative and vascular diseases and also aging (Ozsavci et al., 2007).

The chemistry of DNA damage by several ROS has been well characterized *invitro*, although more information is needed about the changes produced by peroxyl ($RO^{\bullet}_2$), alkoxyl ($RO^{\bullet}$), Ozone (O_3), ONOO−and several of the RNS. Different ROS affect DNA in different ways, e.g. superoxide ($O^{\bullet}_2-$) and H_2O_2 do not react with DNA bases at all OH^- generates a multiplicity of products from all four DNA bases and this pattern appears to be a diagnostic 'fingerprint' of OH^- attack. By contrast singlet oxygen ($^1O_2$) selectively attacks guanine. The most commonly produced base lesion, and the one most often measured as an index of oxidative DNA damage, is 8-hydroxyguanine (8-OHG) (Wiseman and Halliwell, 1996).

Botto et al (2001) reported the presence of chromosomal damage in peripheral blood lymphocytes of 53 patients with coronary ischemia heart disease by using micronucleus test. In the present study we have also observed the high levels of oxidative DNA damage in CHD patients followed by FDR's compared to controls (Table 4).

Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI)

Many studies have demonstrated the role of antioxidants and oxidative stress in cardiovascular diseases. It has been reported that increased intake of antioxidants such as vitamins C and E, protects cardiovascular diseases (Zhang et al., 2014, Subash et al., 2010).

The natural antioxidant system consists of a series of antioxidant enzymes and numerous endogenous and dietary antioxidant compounds that react with and inactivate ROS. The primary antioxidant enzymes include, but are not limited to, superoxide dismutases

(SOD), catalase (CAT) and glutathione peroxidase (GPX). Meanwhile, the nonenzymatic antioxidants include, vitamin C, vitamin E, β-carotene, reduced glutathione and numerous phytochemicals. Cells must maintain their level of antioxidants, often defined as their antioxidant potential, through dietary intake and/or de novo synthesis.

Studies by Khaki-khatibi et al (2012), Surekha et al (2007) and Fazendas et al (2000) have found that TAS levels were significantly lower in CHD patients while the studies by Aksoy et al (2012), Aydın et al (2012) on TOS and OSI are found to be significantly high in CHD patients. Our findings are in consistent with them, and a decreased TAS levels, increased levels of TOS and OSI might be associated with the oxidative stress in CHD patients followed by FDRS compared to controls (Table 4) therefore measurement of TAS, TOS and OSI in body fluids is very important.

C-reactive protein (CRP)

CRP is usually not seen in normal vessel wall and its presence and deposition early in atherosclerotic lesion is preceded by the monocytes appearance. It has been shown that, CRP can stimulate the expression of adhesion molecules and chemokines in human endothelial cells and acts in synergy with lipopolysaccharide to trigger endothelial cells to activate tissue factor production by monocytes. Thus, CRP is not only a marker of inflammation, but also an amplifier of it (Edward et al., 2001).

CRP and atherosclerosis had a link in the beginning as biomarker v/s mediator of atherosclerosis. This dogma has been revisited and suggested that CRP has a direct effect to promote atherosclerotic processes, contributing to the initiation and progression of atherosclerotic lesions. Accumulating evidence suggests that circulating high-sensitivity CRP represents one of the strongest independent predictors of vascular death in a number of settings and it might be a strong biomarker than LDL cholesterol undoubtedly and gives a value to conventional Framingham risk assessment (Koenig et al., 2006).

Pearson's Co-relation

Further we have also analysed the Pearson's correlation coefficient for the correlation between clinical variables of CHD patients and FDRS as represented in Table 5. In the present study Diastolic Blood pressure, Total cholesterol, triglycerides, LDL cholesterol, VLDL, NO, Comet tail length, TAS, OSI and CRP have shown significant correlation at the 0.01 level (2-tailed) with CHD patients while in FDRS Diastolic Blood pressure, triglycerides, LDL cholesterol, VLDL, MDA, NO, Comet tail length, TOS, OSI and CRP have shown significant correlation is at the 0.01 level (2-tailed) (Table 6).

Regression Analysis

The study also analyzed the association of Cholesterol, Triglycerides, LDL, VLDL, MDA and NO against HDL in CHD patients and FDRS (Table 7) by regression and found that the levels of HDL were influenced by Cholesterol, Triglycerides, LDL.

We have also analyzed the association of comet length with Cholesterol, Triglycerides, LDL, VLDL, MDA and NO in CHD patients and FDRS and found that the influence of NO is more than that of MDA (Table 8). Nitric oxide or, more likely, reactive products derived from it, such as NO_2, ONOO−, N_2O_2 and HNO_2 with the potential to produce nitration, nitrosation and deamination reactions on DNA bases might be one of the cause for DNA damage as observed as comet tails in the present study.

Further we have also noticed that the decrease in levels of TAS was influenced by TOS and OSI (Table 9) in CHD patients and FDRS which indicates the role of oxidative stress in the pathogenesis of CHD and it may help in prediction of disease at early stage.

CHD develops over the lifespan of an individual. As people age the more likely they are to develop CHD and suffer a fatal heart attack. After 40 years of age, the lifetime risk of developing CHD is 49% for men and 32% for women. More than four out of five or 81% of the people dying from CHD are 65 years of age or older (Roger et al.,., 2012).

Diabetes mellitus magnifies the risk of cardiovascular morbidity and mortality. The presence of diabetes produced a fourfold to fivefold increase in the risk of CHD. Besides the well-recognized microvascular complications of diabetes, such as nephropathy and retinopathy, there is a growing epidemic of macro-vascular complications, including diseases of coronary arteries, peripheral arteries,

and carotid vessels, particularly in the burgeoning type 2 diabetic population (Beckman et al., 2002).

Recent epidemiological studies have reported that having a family history of CHD is an autonomous risk factor for CHD development. In particular, having a family history of CHD is associated with 2 to 3 fold increase in risk of developing cardiovascular disease. Furthermore, maternal family history of CHD may even predict future cardiovascular events more strongly than a paternal family history (Scheuner et al., 2006).

Epidemiologists predict that the epidemic of obesity and its public health consequences will continue to increase over the next several decades, affecting both the developed and developing worlds. An abdominal pattern of fat distribution produces the most profound metabolic abnormalities and is associated with an increased risk of atherosclerotic cardiovascular disease (Nissen et al., 2008).

Hypertension (HT) is a major risk factor for coronary heart disease (CHD). Among the numerous risk factors associated with CHD, HT plays a major role given its high frequency and its physiopathogenesis. Thus, roughly 15% of the general adult population manifest HT with a net male predominance, and 25% of patients with CHD have HT. CHD is the first cause of morbidity and mortality in hypertensive patients (Baguet and Mallion, 2005).

Cigarette smoking is a powerful independent risk factor for sudden cardiac death in patients with CHD; smokers have more risk about 2-4 times than non-smokers. Cigarette smoking also acts with other risk factors to greatly increase the risk for coronary heart disease.

Many epidemiological studies have indicated a protective role for a diet rich in fruits and vegetables against the development and progression of CHD. Physical inactivity and unhealthy eating contribute to CHD conditions (Ignarro et al., 2007).

The main underlying pathology of atherosclerosis, a process of cumulative deposition of Total Cholesterol and LDL in the arteries supplying blood to the heart that eventually leads to impaired or absent blood supply and myocardial infarction or stroke. Consistent and compelling evidence has demonstrated the association between lipoprotein-associated lipid concentrations and cardiovascular disease incidence worldwide as high concentrations of LDL cholesterol are associated with increased risk of CHD, while high concentrations of HDL cholesterol are associated with decreased risk of CHD (Law et al., 2003).

In most populations, serum total cholesterol increases as age increases. In men, this increase usually levels off around the age of 45 to 50 years, whereas in women, the increase continues sharply until the age of 60 to 65 years. Like serum cholesterol, blood pressure also tends to increase with age, and more prominently in women than in men (Jousilahti et al., 1999).

Conclusion

The aetiology of CHD is multifactorial. It is the result of interaction between genetic, lifestyle and environmental factors. Monitoring the epidemiology and clinical risk factors of CHD is an important component in the pathogenesis of CHD. Despite continued improvements, CHD remains the leading cause of death in India. Epidemiology and clinical data provide the most complete and up to date indicators of the burden of CHD in India.

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