The Riskcorn Study

A study of the risk factors for coronary artery disease in a Pakistani population

Sania Nishtar S.I., FRCP, Ph.D

Heartfile

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A Heartfile Publication

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Suggested citation: Nishtar S. The Riskcorn Study. Islamabad, Pakistan; 2006.

Cover and Layout: Yasir A. Mirza, Heartfile
Consultant Editor: Shahina Maqbool

The author acknowledges the contributions of the following with profound gratitude

Collaborators in United Kingdom:
Dr. Jaswinder Gill, Consultant Cardiologist, Department of Cardiology; Dr. Anthony S. Wierzbicki, Senior Lecturer in Chemical Pathology; Prof. Michael S. Marber, Professor of Cardiology; Michelle Lambert-Hammill and Peter J Lumb, Research Assistants, Department of Chemical Pathology; Charles N. Turner, Research Assistant, Department of Pediatrics at the St. Thomas' Hospital, London, United Kingdom.

Collaborators in Pakistan:
Prof. Mohammad Afzal Mattu, Professor of Cardiology; Prof. Naseer Ahmed, Professor of Cardiology and Dr. Iqbal Saifullah Khan, Pakistan Institute of Medical Sciences, Islamabad, Pakistan and Maj. Gen. Masud ur Rehman Kiani, Commandant and Maj. Gen. Ashur Khan, Chief of Cardiology at the Armed Forces Institute of Cardiology, Rawalpindi, Pakistan.

Members of the study team at Heartfile:
Dr. Ayesha Ehsan, Dr. Asma Badar, Dr. Rehana Ahmad, Dr. Adeela, Dr. Samia Ali, Rehana Hamid, Amra Qayyum and Yasir Abbas Mirza.

The Pakistan Medical Research Council for the seed funding for this Study; Dr. Anthony S. Wierzbicki, Department of Chemical Pathology, St Thomas' Hospital, London, for supporting the biochemical analysis and the World Health Organization, Pakistan office, for its support for printing of this Monograph.

Completed in **2000**, RISKCORN was Heartfile's first research project and formed both, the evidence for its initial scope of work in the area of health communication as well as the scientific basis of the organizational standpoint on primary and secondary prevention issues. The Study also provided important insight into the subsequently launched and Heartfile-led National Action Plan for the Prevention and Control of Non-Communicable Diseases and Heath Promotion in Pakistan. Today, as the organization focuses on mainstream health policy, systems, and planning issues, RISKCORN remains a reminder of the value of indigenously derived evidence for planning and decision-making.

Sania Nishtar
2006, Islamabad, Pakistan

Précis

It is not widely realized that at present, developing countries contribute a greater share to the global burden of CVD than the developed countries and nothing less than an explosion in this disease burden is projected over the coming years. Located in South Asia, Pakistan has a population of 140 million, of which 64% is rural; surveys in Pakistan indicate high prevalence of cardiovascular disease risk factors with over 30% of the population over 45 years of age affected and call for aggressive preventive strategies.

Clearly, the first step in addressing this issue would be to identify the risk factor profile of this population; this must include research that serves to inform policy and subsequently, the initiation of preventive programs, which could control the CVD epidemic through cost-effective strategies that are feasible and hold the promise of early impact. However, setting goals for preventive initiatives necessitates the definition of the risk factor profile of a population and for the Pakistani population, absence of relevant data made this difficult. Ideally, efforts at uncovering the risk factor profile should be undertaken in a well-designed multicentre prospective cohort design; however, issues of time, resources and time lag made this impractical. Similarly, the 10-20 year lag time for results from such studies makes an urgent case for short to medium term strategies to counter the impending CVD pandemic. Given these constraints, a hospital-based case-control study was undertaken.

This study was the first-ever study of coronary risk factors in a Pakistani population, examining risk factors predisposing to occlusive CAD in a matched case-control study of patients presenting with chest pain to two tertiary referral sites in Pakistan. Four hundred patients were recruited with ethical consent who underwent coronary angiography from 1998-2000 for presenting symptoms suggestive of CAD. Two groups were selected based on the presence of significant CAD as defined by 50% stenosis in one or more coronary arteries and an age and sex matched control group, presenting with similar symptoms but with no angiographic evidence of disease. A detailed cardiovascular risk profile was obtained through a structured interview; this included data on demographic and socio-economic status and lifestyle, personal and family history of CAD. The components of the questionnaire were compiled with the use of previously validated questions included in other studies. Several techniques of observation were also used as methods of data collection; these included triplicate measurement of blood pressure using mercury sphygmomanometer, measurement of waist-hip-ratio and total body fat estimation by the OMRON BF 300 impendence system. Fasting blood samples were obtained for determination of biochemical risk factors including baseline renal function, liver function, glucose, insulin, lipid and apolipoproteins, lipoprotein (a), homocysteine, C-reactive protein, and fibrinogen. Biochemical analytes were measured by automated methods on Cobas Mira and Fara 2 analyzers, Behring BN2 nephlometer and the Corning ACS 180 immunoassay system. Insulin resistance was calculated by the HOMA-R method 4. Angiograms were also quantified for the extent and severity of CAD by the Gensini scoring system by observers blinded to other clinical details; this method assigns a different severity score

depending on the geometrically increasing severity of lesion, the cumulative effects of multiple obstructions and the significance of their geographic locations.

Statistical analysis was initially conducted between cases and controls. Univariate analysis using conditional logistic regression of each variable was carried out and matched odds ratios with 95% confidence interval were obtained. Conditional multiple logistic regression technique was used for building the final model; analysis was conducted by the best subset selection technique. In addition to case-control analysis, linear regression and correlation were also used by using baseline data or log transformed data depending on whether individual analytes showed a Gaussian distribution, to determine the relationship of Gensini scores and the exposure variables measured on the continuous scale and the nature of the relationship. The statistical significance of the relationship was computed by calculating p-values and regression coefficients. Statistical analyses were conducted using SPSS 7 for Windows 98 and GB Stat 7.0 (Dynamic Microsystems, Silver spring, Maryland, USA).

In univariate conditional logistic regression analysis, significant associations of disease were found with several risk factors; these included current and past status of smoking, passive smoking, exposure to environmental tobacco smoke as a result of the spouse smoking, lifetime cigarette exposure, socio-economic stress, sedentary habits, history of diabetes and high blood pressure, consumption of fatty foods, family history, fat percentage, waist circumference, waist-hip ratio, low apolipoprotein A1, low HDL, lipoprotein (a), glucose, insulin, insulin resistance, CRP, sialic acid, creatinine and bilirubin. In multiple conditional logistic regression, however, significant associations were found only with low HDL, family history, C reactive protein, and waist-hip ratio after adjusting for all other factors in the model. In addition, linear regression analysis of Gensini scores with risk factors showed that the risk of occlusive CAD also correlated with age, the duration of diabetes, waist-hip-ratio, low HDL, lipoprotein (a) and creatinine.

In this study, family history has emerged as one of the strongest predictors of CAD. The underlying mechanism through which family history can be associated with CAD could either be lipid related or linked to the insulin resistance syndrome. There are several familial lipid disorders including familial hypercholesterolaemia (FH), familial combined hyperlipidaemia (FCH), familial hypoalphalipoproteinaemia (FHA) and familial dyslipidaemia which may be involved in mediating this risk; analysis of data, however, reveals that this is unlikely since neither LDL nor triglycerides were strongly associated with coronary risk in this study; elevation of either LDL or triglycerides is one of the components of the above mentioned familial lipid disorders. On the other hand, the strong association with family history may be linked to Lp(a). Plasma levels of Lp(a) are 90% genetically determined, therefore, it appears to be a highly heritable trait. A strong genetic link is also known to exist for the insulin resistance syndrome; most of the hallmark abnormalities of the insulin resistance syndrome were strongly associated with CAD in this study. These include glucose, waist-hip ratio, low HDL, low apo A, insulin and insulin resistance; small LDL and apo B were also seen to play a marginal role. The results of this study also support the hypothesis that inflammation mediates the increased coronary risk associated with insulin resistance as was evidenced by a strong correlation of markers of inflammation with markers of insulin resistance. The association of disease with the duration of diabetes and creatinine help to further highlight the role of this genetically inherited metabolic complex in the pathogenesis of CAD.

Environmental factors were also seen to influence coronary risk significantly and reinforced the earlier impressions about the importance of these risk factors based on studies in White populations. These include smoking, diet and physical activity. Favourable environmental and lifestyle choices can also be seen to significantly influence coronary risk through its interaction with the insulin resistance syndrome.

This is one of few studies performed in native South Asian populations as opposed to migrants to developed countries and the first study to use quantitative angiographic data in this population as opposed to cross-sectional epidemiological cohort study methods. It differs from other studies in showing that despite a predominantly meat-eating diet, populations in Pakistan have similar total cholesterol and LDL to Hindus from North India and lower values than those reported from Southern India, where there is a high prevalence of vegetarianism but also a high saturated fat intake. The study also found marked differences in anthropomorphic and insulin resistance associated variables between cases and controls. Further biochemical analysis will be conducted on the stored blood samples for risk variables associated with insulin resistance and once genetic risk factors for the metabolic syndrome have been clarified, this group will prove ideal to assess the contribution made by these risk factors in a native South Asian population.

The public health significance of the data gathered is that despite levels of LDL considered adequate as targets in Europe (3 mmol/l) and the USA, (2.5 mmol/l), many patients in Pakistan show the presence of significant established CAD. In fact, in this population, HDL is a far stronger risk factor. This suggests that drug recommendations based on the European/American practice recommending the use of statins as first-line agents may not be entirely correct and highlights the need to redefine the currently practised therapeutic approach to CAD management in this population to fit local needs.

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Acronyms

ACE	angiotensin converting enzyme
AFIC	armed forces institute of cardiology
AMI	acute myocardial infarction
Apo A1	apolipoprotein A1
Apo B	apolipoprotein B
ATP	adult treatment panel
AST	aspartate aminotransferase
ALT	alanine aminotransferase
BMI	body mass index
CAD	coronary artery disease
CAM	cell adhesion molecule
CCB	calcium channel blocker
CHD	coronary heart disease
CMV	cytomegalovirus
Cpn	chlamydia pneumoniae
CRP	c-reactive protein
CK	creatine kinase
DALY	disability adjusted life years
ETS	environmental tobacco smoke
FFQ	food frequency questionnaire
GGT	gamma glutamyltransferase
GNP	gross national product
GDP	gross domestic product
HDL	high-density lipoproteins
IHD	ischemic heart disease
IRS	insulin resistance syndrome
JNC	joint national committee
LDL	low density lipoprotein
Lp(a)	lipoprotein (a)
MOR	matched odds ratio
NCD	non-communicable diseases
NEFA	non esterified fatty acid
OR	odds ratio
PIMS	Pakistan institute of medical sciences
PAI 1	plasminogen activation inhibitor
RCT	randomised controlled trial
SES	socio-economic status
SMR	standardized mortality ratio
TC	total cholesterol
TG	triglycerides
tHcy	total homocysteine
t-PA	tissue plasminogen activator

Glossary

Beeri: a handmade form of cigarette made by the consumer by wrapping tobacco in temburni leaves; although the quantity of tobacco used is smaller than in a cigarette, the tar yield, nicotine content and concentration of carcinogens released exceed that of an average cigarette.

Chapaati: thinned out pita bread made out of un-risen whole grain flour approximately the size of a quarter plate, cooked on a flat skillet without any oil and is eaten as staple bread in Pakistani households.

Firni/kheer: Pakistani rice pudding usually made with full cream milk.

Ghee: a form of cooking fat, which may be obtained from dairy sources or vegetable oils. The commercial form of ghee available in Pakistan is predominantly derived from the hydrogenation of vegetable oils and is known as "banaspati ghee"; this form of ghee is high in trans-fatty acids. On the other hand, ghee is also derived from dairy sources; this form resembles butter oil in being obtained by the clarification of butter, but for butter oil, clarification is done below 80 degrees C in a vacuum whereas for ghee, temperatures of 100-120 degrees C are used; heating imparts to ghee its characteristic aroma.

Halwa: South Asian sweetmeat made by prolonged cooking of equal parts of saturated fat and a seasonal vegetable.

Hukka: device for smoking tobacco which is alike the middle eastern "hubble bubble"; tobacco is heated in a pot and is made to pass through water cisterns before being inhaled through a long tube; the device is used for communal smoking.

Mithai: commercially available traditional South Asian sweetmeats.

Naswar: orally used snuff.

Pakoras: deep fat fried balls made of chick-pea flour and an assortment of chopped vegetables.

Pan: a mixture of areca nut and slacked lime wrapped in betel leaf in which tobacco is optionally added.

Paratha: the same as a chapaati, except for the addition of substantial amount of cooking fat both within the dough and fried; traditionally eaten for breakfast.

Roghni nan: bread made out of risen whole-wheat flour with the addition of fat in the dough, approximately the size of a dinner plate, cooked in a traditional oven.

Samosas: deep fat fried triangular pockets of thin white flour sheets stuffed with cooked spicy meat or potatoes.

Tandoori nan: bread usually made out of risen whole-wheat flour, approximately the size of a dinner plate, cooked in a traditional oven without the addition of fat.

INTRODUCTION

Introduction

1.1 BACKGROUND

As highlighted in reports by the WHO[1] and the World Bank,[2] the Victoria,[3] Catalonia[4] Singapore[5] and Osaka[6] Declarations and from the Murray and Lopez data,[7] cardiovascular diseases (CVD) are recognized as the major cause of mortality and morbidity in the industrial world.[8] Recent studies indicate that countries in transition and the developing world are also currently or in the near future going to face the same or an even higher burden of CVD, a phenomenon known as the impending global pandemic of cardiovascular diseases.[9] This disease trend has been extrapolated from existing data based on what has been termed the Epidemiological Transition. This term was coined to describe the shift in the disease spectrum from communicable to non-communicable diseases. This change has already occurred in the developed world; the economies in transition are in the throes of the epidemiological shift and now it is the turn of the developing world. It is not widely realized that at present, the developing countries contribute a greater share to the global burden of CVD than developed countries and nothing less than an explosion in disease burden is projected over the coming years. In the year 1999, 16,970,000 people died due to cardiovascular diseases the world over, which accounts for 30.3% of all the deaths worldwide.[1] More than 50% of these deaths occurred in the developing countries.

South Asia composed of the countries of Pakistan, India, Bangladesh, Sri Lanka, Nepal, Bhutan and Maldives is fast emerging as one of the poorest, the most illiterate, the most malnourished, the least gender-sensitive, indeed, the most deprived region in the world.[10] According to World Bank $1 a day estimates, out of the 1.3 billion people in the world who live below the poverty line, 515 million (40%) live in South Asia, even though the region accounts for only 23% of the global population.[11] This gap is also evident by comparing the South Asian GNP per capita of US $393 with $1250 for rest of the developing world. The annual population growth rate of South Asia is about 2% compared with 1.8% for rest of the developing world. For this vast mass of mankind, the average public sector expenditure on health (as a percentage of the GDP) was 0.85% in 1997 compared with 2.0% for rest of the developing world whereas the per-capita health expenditure has recently been reported at US $11-26 for various South Asian countries.[12] Additionally, for every dollar of central government expenditure spent on social sectors, $8.65 is spent on defence and debt servicing in Pakistan, and $3.12 in India, further highlighting the economic and political considerations that constrain the health care scenario in the region.

Located in South Asia, Pakistan has a population of 140 million[13] of which 64% is rural, and 75.9% below the age of 35 years. For this vast population, there are 18 cardiology departments, 384 cardiologists and 19 cardiac surgeons. Surveys in Pakistan indicate high prevalence rates of risk states for cardiovascular disease, with over 30%[14] of the population over 45 years of age, affected and call for aggressive preventive strategies. Pakistan's economy and health resources, on the other hand, translate into tough health priorities, where communicable, nutritional and maternal and child health issues are prioritised. With a

per capita income of $500, privately funded health care falls far short of the bare minimum and with the total health expenditure at 0.8% of the GNP,[15] the public sector is far from widening its health sector outreach for curative cardiovascular care. With cardiovascular diseases now a major health concern, the health economics of these countries are likely to be stretched beyond already meagre limits. Fortunately, heart diseases are preventable to a very large extent and therefore the major thrust in overcoming these at a global level has been on prevention. Even the most developed countries in the world have not found technology and treatment as a solution to this rising epidemic, but have found prevention as the only answer.

For preventive strategies, the definition of the risk factor profile of a population to set targets for intervention and goals for preventative initiatives is crucial. For the South Asian population, this has been complicated by several factors. Lessons learnt from the expatriate coronary risk highlight increased mortality and morbidity trends that are partially unaccounted for, by the traditional risk factors.[16,17] In native populations, prevalence of risk factors and causal and temporal relationships for coronary artery disease (CAD) have not been well established and against the backdrop of these current gaps in our knowledge, no work has been done on the role of novel risk factors. The risk factor profile of this population may also be continuously evolving as both the emigrant and the indigenous populations are undergoing rapid urbanization and acculturation of western lifestyles and behaviours.

It may be true that the overall high CAD risk cannot be explained on the basis of conventional risk factors alone as the strength of association of risk factors with CAD varies in different ethnic groups. Conventional risk factors for CAD such as smoking, high blood pressure, dyslipidaemia and diabetes are however, highly prevalent in some South Asian ethnic subgroups[18] and will remain the key, if not the only elements of the risk factor model in this population. The identification of other emerging or protective risk factors is also important so that new approaches to the prevention of CVD may be developed because a single risk factor model and prevention strategy is not applicable to all populations. It seems likely that in South Asians, ethnicity, or some unmeasured or as yet, unknown proximate and remote risk factor linked to ethnicity, not included in the typical classical high risk composite seen in the Caucasian model, might mediate the effect of atherosclerosis on the risk of clinical cardiovascular disease. There is therefore a need to re-think the approach towards identifying and mediating CVD risk in the Pakistani population, with current research also focusing on the role of newly emerging risk factors.

Clearly, the first step in addressing this issue would then be to identify the risk factor profile of this population and must include research that serves to inform policy and subsequently the initiation of preventive programs, which could control the CVD epidemic through cost-effective strategies that are feasible and hold the promise of early impact. Health prevention and promotion are the only affordable solutions for the vast populations of South Asia. Classical medical interventions are, and will continue to remain, inaccessible to the majority of the population in the short to medium term.

The ideal model would be a well-designed multi-centre prospective cohort study in the Pakistani population. However, issues of cost, time and institutional capacity make such an effort unlikely in the near future. Similarly the 10-20 year lag time for results from such studies makes an urgent case for short to medium term strategies to counter the impending CVD pandemic. It is here, that well designed case-control studies can build upon data coming

in from numerous cross sectional studies on both the indigenous as well as the emigrant South Asian populations. The study described within this thesis "The study of the risk factors for coronary artery disease in a Pakistani population" (RISKCORN) is therefore an important step in identifying the coronary risk factor profile of the Pakistani population.

In Pakistan, population-based risk factor prevalence data is available but no attempt has been made to identify risk factors amongst those with established CAD, particularly in cases with angiographically established disease. This study will help in identifying persons who are at special risk of CAD with implications for preventive strategies; it will also add to existing knowledge about the aetiology of CAD in the Pakistani population, in addition to obtaining information that will be a basis of a decision on the utilization of resources.

1.2 THE PRESENT STUDY

RISKCORN addresses a broad array of known and postulated risk factors and attempts to show epidemiological relationships between the presence or absence of CAD and the proximate and remote determinants of CAD risk factors.

Beginning December 1998, through to September 2000, in a hospital based case-control study design, 200 Pakistanis with documented CAD as evidenced by angiography were enrolled. After ascertaining their eligibility for enrolment and a written consent, the patients were questioned to fill out the study questionnaire on different aspects of lifestyle, following which, standardized measurements and documentation of anthropometric data was carried out. Finally, blood samples were drawn and aliquoted according to predetermined laboratory protocols. Similarly, an equal number of healthy, age and sex-matched controls, who were known not to be suffering from CAD as evidenced by normal coronary angiograms, were enrolled. The cases and controls were drawn from a Pakistani population in two angiographic centres in Pakistan. Their blood samples were stored at -70 and -20 degrees C, and were shipped in three consignments, September 1999, March 2000, and October 2000 on dry ice to the department of Chemical Pathology, St Thomas' Hospital London, where biochemical analysis was performed.

1.3 HYPOTHESIS

The distribution of cardiovascular risk factors for angiographic coronary heart disease differs in a native Pakistani population from that observed in other Asian and Caucasian populations.

2 REVIEW OF LITERATURE

Review of Literature

2.1 RISK FACTORS FOR CORONARY ARTERY DISEASE

Decades of research have demonstrated that coronary artery disease (CAD) has multi-factorial causes and the term "risk factor" was first used in this context. A risk is a trait predicting the possibility of getting a disease. The identification of risk factors in the context of CAD has important practical implications as it forms the basis of risk reduction strategies within populations. Several criteria have been used as guidelines to judge whether an epidemiological association could reflect a role for a specific given risk factor. A risk factor must fulfil most, if not all the criteria of causality; strength of association (high relative risk or odds ratio); consistency of association (over many studies); temporal relation (cause preceding the effect); dose-response relation (greater the exposure, higher the risk); biological plausibility (coherence); independence (risk is evident even after adjustment for other risk factors); experimental evidence and, very importantly, evidence from human studies. An over view of the individual risk factors for CAD is presented.

2.1.1 Hypertension

The positive relationship between systolic and diastolic blood pressure and cardiovascular disease has long been recognized. This relationship is strong, continuous, graded, consistent, independent, predictive, and aetiologically significant for those with and without CHD.[19] The risk rises progressively with blood pressure, with no evidence of a threshold or a J-shaped relationship. In the follow-up of the Multiple Risk Intervention Trial (MRFIT), relative risk of coronary events increased from 1.0 in those with systolic blood pressure <120 mm Hg and diastolic blood pressure <80 mm Hg to 3.2 in those with isolated diastolic blood pressure >100 mm Hg, to 4.2 in those with isolated systolic blood pressure >140 mm Hg, and to 4.6 in those with combined systolic and diastolic hypertension (>160/100 mmHg).[20] The risk of CVD, in hypertensive individuals increases when other risk factors are concomitantly present, such as cigarette smoking, hyperlipidaemia and diabetes.[21]

2.1.2 Tobacco use

The relationship between smoking and CVD is unequivocal.[22] Smoking ranks as the largest preventable cause of CAD,[23] increasing cardiovascular mortality by 50% and doubling the incidence of CVD.[24] A smoker dies three years earlier than a non-smoker and

10 to 15 years earlier, if a smoker is known to be at high risk for coronary disease. Smoking also increases the risk of sudden cardiac death.[25]

The constituents of tobacco smoke increase smooth muscle cell proliferation, platelet aggregation, adherence of platelets to endothelium,[26] plasma viscosity and levels of fibrinogen and factor VII.[27] Levels of plasminogen are reduced in smokers.[28] In addition, carbon monoxide reduces the oxygen carrying capacity of blood,[29] thus limiting oxygen availability and exacerbating myocardial ischemia.[30] In response to smoking, elevations in VLDL and reductions in HDL also occur.[31] Nicotine causes the heart rate, blood pressure and cardiac output to increase and exacerbates vasoconstriction particularly in the coronary arteries,[32] with a consequent increase in myocardial oxygen demand and a reduction in oxygen availability. Nicotine can also sensitize the myocardium, rendering it more irritable and susceptible to the development of malignant arrhythmias, and therefore cigarette smoking significantly increases the risk of sudden cardiac death.[25] The effectiveness of many commonly prescribed cardiac medications also decreases in the face of continued smoking.[33]

Those who have never smoked, but who have been exposed to environmental tobacco smoke (ETS) are also at increased risk of developing CVD; the increase in relative risk is calculated to be between 1.2 and 1.3.[34,35] The cardiovascular effects of smokeless chewable tobacco are also known to be similar to those of cigarette smoking,[36] however, the age adjusted relative risk of dying from CVD is lower with smokeless tobacco than with tobacco smoking.[37]

Smoking is also known to increase the risk of athrothrombotic events, increasing plaque thrombogenicity and plaque tissue factor expression.[38] As a consequence of all these factors, smoking plays a pivotal role not only in the acceleration of atherosclerosis, but also in the pathogenesis of acute coronary events.

2.1.3 Dyslipidaemia

2.1.3.i Total Cholesterol: twenty five year follow up of the seven countries study, compared the relationship between total cholesterol and long term mortality from CAD in different cultures across 7 countries involving over 12,000 men, measuring total cholesterol at the baseline and at 5 and 10 year follow ups. The results showed that across cultures, cholesterol was linearly related to CAD mortality and that the relative increase in CAD mortality rates with given cholesterol increase was the same. The large difference in absolute CAD mortality rates at a given cholesterol level, however, indicates that other factors, such as diet, that are typical for cultures with low CAD risk are important with respect to prevention.[39]

2.1.3.ii LDL cholesterol: in general, populations with high mortality from CAD have a high average level of plasma cholesterol and where the average plasma cholesterol is less than 5 mmol/l, CAD does not occur on a mass scale, even despite the common occurrence of other risk factors.[40]

The conclusion that plasma LDL should be lowered in patients with CAD is based on several randomized clinical trials in which the overall results are concordant. With LDL lowering as attained in the trials, i.e. reduction of approximately 35%, death would be reduced by 25% and clinical events by somewhat more. (STARS,[41] MARS,[42] CCAIT,[43] HARP,[44] PLAC 1,[45] MASS,[46] SCRIP,[47] 4S,[48] CARE,[49] LIPID,[50] AFCAPS/TexCAPS,[51] WESCOPS[52]) Mechanisms independent of LDL lowering may also play an important role in the clinical benefits conferred by LDL lowering drugs and may in future broaden their indication from lipid-lowering to anti-atherogenic agents.[53] This includes improvement in endothelial function,[54] reduction in the proliferation of macrophages[55] and favourable effects on thrombosis and inflammation.[56]

Notwithstanding that CAD risk rises progressively with increases in total and LDL cholesterol, most patients with CAD do not have markedly elevated levels of either. For example, in a survey, it has been demonstrated that only 23% of patients with CAD have total cholesterol levels greater that 6.2 mmol/l (the 75th percentile of the population), and only 26% have LDL levels greater than 4.2 mmol/l (the 75th percentile of the population).[57] This further highlights the role of multiplicative risk arising from a combination of risk factors and identifies that the largest proportion of CVD events in any community arises from persons who have modest elevations of many risk factors than from individuals with marked elevation of a single risk factor.

The strong association between CAD and dyslipidaemia has often overshadowed the role of non-lipid risk factors, including smoking, hypertension, obesity, diabetes and impaired glucose tolerance and has even led to questioning the importance of these risk factors in the presence of a favourable lipoprotein profile. Recently, substantial effects of these non-lipid risk factors on the extent and severity of coronary and aortic atherosclerosis have been demonstrated, even in the presence of a favourable lipoprotein risk factor profile; this and other data supports the need to control all cardiovascular risk factors.[58]

2.1.3.iii Apolipoprotein B: apolipoprotein B is the predominant apolipoprotein of LDL. In many cases of CAD, apolipoprotein B may be increased despite the presence of normal levels of LDL, this condition is known as hyperapobetalipoproteinaemia (hyperapoB). In this condition, LDL particles are cholesterol depleted and are relatively protein rich. A possible explanation of the decreased cholesterol to protein ratio in hyperapoB might be a shift in the spectrum of LDL towards smaller denser LDL.[59]

2.1.3.iv Small LDL: there is increasing evidence to suggest that small dense LDL is a strong risk factor for CAD.[60] Higher frequency of smaller LDL has also been observed in patients with premature CAD[61] and is found to be a statistically significant risk factor in patients with familial hypercholesterolaemia.[62] Small dense LDL is cholesterol ester poor and is triglyceride enriched, and is shown to have a lower affinity with the apoB receptor; therefore it appears that the shift towards smaller and denser particles may alter the metabolism of LDL in such a manner that it becomes more atherogenic.[63] LDL size also correlates negatively with plasma triglyceride[64] and insulin levels;[65] therefore, it appears that there is also a close association between small dense LDL and insulin resistance.

2.1.3.v HDL: the risk of CAD is inversely related to HDL levels. Low HDL is a strong independent predictor of CAD; the evidence for this association is derived from epidemiological studies.[66,67] There is also evidence for this association in studies linking HDL level to the rate of progression of CAD and the extent of CAD on angiography.[68] Low HDL both modifies the goal for LDL lowering therapy and is used as a risk factor to estimate the 10-year risk for CAD in the ATP [111] guidelines.69 Low HDL cholesterol is also common in patients with premature CAD; familial hypercholesterolaemic patients with CAD show significantly lower values of mean plasma HDL cholesterol and a higher total/HDL cholesterol ratio compared with familial hypercholesterolaemic subjects free of CAD.[70] Low HDL is always associated with abnormalities of lipoprotein metabolism which are atherogenic and is also associated with insulin resistance, diabetes and hypertension which further increase the risk. It has been suggested, that the link between low HDL levels and atherosclerosis may depend on up regulation of inflammatory mechanisms putatively induced by low HDL. This hypothesis has been supported by the recent demonstration of elevated C-reactive protein levels in familial hypoalphalipoproteinaemia, in the absence of signs and symptoms of local or systemic inflammation or recurrent disease.[71]

One of the best predictors of future CAD events is the ratio of total cholesterol to HDL ratio. The ratio reflects the fact that, even at low plasma total cholesterol levels, low HDL concentrations significantly increase the risk of future CAD events, while high HDL is protective even if total (and LDL) plasma cholesterol levels are high.[72,73] Increases in the ratio are particularly significant if accompanied by higher triglyceride levels.

2.1.3.vi Triglycerides: earlier, it was unclear whether hypertriglyceridaemia was an independent risk factor for CVD.[74] Univariate analyses almost invariably identified triglyceride levels as a significant risk factor while multivariate analyses almost invariably did not. The lack of epidemiological evidence conformed to the biological reality; cardiovascular risk is not commonly associated with the most elevated triglyceride levels as is seen in case of disturbances of chylomicron metabolism. By contrast, mild to moderate elevations in plasma triglyceride levels are commonly found in patients with CAD. Mild hypertriglyceridaemia, particularly when combined with low HDL is at least, as powerful a predictor of CAD as isolated high LDL.[75,76] This combination is often seen in the metabolic syndrome with visceral obesity, hypertension and insulin resistance. A meta-analysis of 17 prospective population based studies indicates that plasma triglycerides are a risk factor independent of HDL levels.[74]

There is, however, no clinical trial evidence that lowering triglyceride levels changes cardiovascular risk. Limited clinical trial data suggest that, fibrates may reduce cardiovascular risk, but multiple mechanisms may be responsible, including their effects on triglyceride and HDL levels and LDL remodelling, making interpretation difficult.[77,78]

2.1.3.vii Lipoprotein (a): lipoprotein (a) is a cholesterol-rich lipoprotein composed of a LDL particle linked to apolipoprotein A, a protein with a terminal amino acid sequence similar to that of plasminogen. Lp(a) is an independent risk factor for CAD with a relative risk of 2.7 at levels >20 mg/dl.[79] Numerous cross-sectional and case control studies have

demonstrated that elevated Lp(a) concentrations are associated with premature atherosclerosis.[80]

However there is great debate over the mechanisms by which Lp(a) is associated with CAD. It is also unclear whether it contributes to atherogenesis or thrombosis or both; Lp(a) has also been suggested as a marker for vascular injury which further complicates the issue. Lp(a) accumulates in atherosclerotic plaques,[81] promotes thrombosis[82] and foam cell formation,[83] stimulates smooth muscle cell proliferation,[84] impairs endothelium-dependent vasodilatation[85] and promotes monocyte chemo-attractant activity in human vascular endothelial cells.[86] Oxidized Lp(a) has been shown to increase vascular endothelial cell production of plasminogen activator inhibitor-1 (PAI-1).[87] Plasma Lp(a) levels are also known to correlate with reduced acetylcholine-mediated coronary vasodilatation in human coronary arteries examined during diagnostic coronary angiography.[88]

The inconsistent nature of the association between Lp(a) and CAD across populations may be due to different factors. These include lack of standardization of assays for this lipoprotein and problems associated with comparison of the mean values; Lp(a) is not normally distributed and because it is markedly skewed at the lower end of the range, mean Lp(a) values may be potentially misleading. In Caucasians, Lp(a) levels have a highly skewed distribution,[89] whereas the phenotype-genotype correlation for Lp(a) in other populations is more variable with a Gaussian distribution in Africans. Plasma concentrations vary from 1 mg/dl to more than 100 mg/dl in different individuals. In a single individual however, plasma Lp(a) concentration is remarkably stable over time and is not greatly influenced by age, sex, diet or most pharmacological interventions that significantly alter plasma concentrations of other lipoproteins.[90,91]

The pathogenicity of Lp(a) is dependent on LDL-cholesterol levels; LDL-cholesterol concentration modulates the ability of Lp(a) to promote atherosclerosis. In recent studies, plasma Lp(a) concentrations predicted the risk of early atherogenesis in a dose-dependent fashion, with this association being confined to subjects with LDL cholesterol levels above the population median (3.3 mmol/l); apo A phenotypes of low molecular weight emerged as one of the strongest risk predictors of advanced stenotic atherosclerosis, especially when associated with high Lp(a) (OR 6.4; 95% C.I. 2.8, 14.9).[92] CAD risk in patients with high Lp(a) is also much higher in patients with low HDL.[93]

There is strong evidence to suggest that Lp(a) concentrations in blood are genetically determined through autosomal dominant transmission.[94] As Lp(a) is 90% genetically determined[95] there is limited value of Lp(a) with regard to risk factor intervention; Lp(a) may however, have a role in the identification of patients with premature CAD.[96]

2.1.4 Dietary fat, fatty acids, antioxidants and fibre

Epidemiological and experimental studies have identified many foods and nutrients which protect against or promote CAD. These have been enumerated in Table 2.1.

Table 2.1: Foods and nutrients identified to be potentially protective against or to promote coronary heart disease or cardiovascular risk factors

Protective	Promotive
Fruit and vegetables	High fat dairy products
Wholegrain cereals	Eggs
Fish	Red and organ meats
Nuts	Cholesterol
Soy products	Trans unsaturated fatty acids
n-3 fatty acids	Saturated fatty acids
Antioxidant nutrients	
Folate	
Dietary fibre	
Alcohol (moderate intake)	

2.1.4.i Dietary modification and CAD: diet may impinge on CAD risk through obesity and increase in serum cholesterol. Dietary alternations can alter both body weight and serum cholesterol and therefore decrease the risk of developing CAD.[97] It is beyond debate that a diet high in fruits, vegetables and wholegrain products reduces CAD risk, whereas a necessary condition for the development of high CAD rate in a population is an eating pattern characterized by high level of energy intake compared with energy expenditure and a high content of cholesterol and saturated fat, total fat, sugar, sodium and alcohol; such a diet includes high intake of animal products, such as meat, eggs and dairy products, and is energy dense. This diet has been used mostly in developed countries at the expense of the intake of wholegrain foods, legumes, vegetables, and fresh fruit and is responsible for the high prevalence of hyperlipidaemia, hypertension and excess weight. By causing individuals to become overweight, this diet also enhances the risk of type 2 diabetes, further adding to the risk.

A recent meta-analysis summarized randomised controlled trials (RCTs) that looked at the extent to which dietary manipulations involving foods or nutrients might be expected to reduce cardiovascular risk. These included replacement of much of the saturated fatty acid content with the polyunsaturated fatty acid, linoleic acid. Results revealed 16% reduction in all cardiovascular events, 9% reduction in cardiovascular mortality and a small non-significant 2% reduction in total mortality. When considering those trials which continued for more than 2 years, the benefit was greater, a 24% reduction in all cardiovascular events.[98] However, greater benefit is apparent when examining the results of multi-factorial trials. In the Oslo Study, saturated fats were replaced as far as possible by wholegrain cereals, vegetables, fruit and some unsaturated fatty acids and oily fish was encouraged; these changes would have been expected to reduce LDL and its oxidation, thrombogenesis, blood pressure and probably also homocysteine levels. A 36% reduction in total mortality and 44% decrease in cardiovascular events in association with a 13% reduction in cholesterol over a 6 year period were observed when compared with the control group.[99] In another multi-factorial dietary intervention study, the intervention diet was intended to reproduce the Mediterranean dietary pattern; the benefits in terms of both cardiovascular events (cardiac death and non-fatal myocardial infarction) and total

mortality were remarkable (Risk ratios 0.27; 95% C.I. 0.12, 0.59 and 0.30; 95% C.I. 0.111, 0.82, respectively).[100]

2.1.4.ii Dietary fat: historically, the 'diet-heart' hypothesis was first presented by Anitschkow in 1913;[101] which subsequently fuelled three lines of research among humans: ecological studies of diet and CVD among large groups of individuals, within-population epidemiological studies relating diet to serum cholesterol and to the risk of CVD, and experimental studies in humans.

Ecological studies included international comparisons of dietary patterns and CVD occurrence in various countries, an example of which is the International Atherosclerosis Project (IAP), which found high correlation coefficients between estimated population-wide dietary fat intake and the extent of atherosclerosis among 21,000 autopsies conducted in 15 countries.[102] The major limitation of ecological studies is that it is impossible to know whether the attributes being correlated actually occur in the same individuals, making these investigations very susceptible to bias. To try to alleviate this problem, investigators have studied dietary intakes and CVD among cohorts from several countries.

The most important example of this strategy is the Seven Countries Study, which showed a very strong correlation between saturated fat intake and five-year CAD mortality ($r=0.84$), although the relationship with total fat intake was less impressive ($r=0.40$). [103] The strong relationship between saturated fat intake and CAD has recently been shown to extend through 25 years of follow-up of the Seven Countries Study.[104]

Another example of ecological studies involves the follow-up of migrant populations; examples of this are the Ni-Hon-San study,[105,106] which compared CVD risk factors and IHD deaths among Japanese men living in Japan, Hawaii and San Francisco. The comparison of cohorts of individuals of similar ancestry removed the potential confounding effect of genetic factors involved in the diet-CVD relationship. The percentage of calories from saturated fats differed substantially among the cohorts: 7%, 23% and 26% for the cohorts from Japan, Hawaii and San Francisco, respectively. The five-year IHD mortality rates paralleled the dietary fat intake: 1.3, 2.2 and 3.7 per 1000, respectively.

Although ecological studies suffer from many flaws, such as not being able to control for the confounding effects of other major risk factors, they do provide evidence that supports the diet-heart hypothesis; to establish whether diet is a risk factor at the individual level, studies within defined populations are required. Within-population studies allow direct measurement of diet, other risk factors and the outcome of interest in individuals and provide an opportunity to adjust for potential confounding variables; however, these studies have inherent limitations in studying the impact of diet on CVD in homogeneous populations. Firstly, the probability that significant associations can be found at the individual level is less. Secondly, because of the efforts and costs involved in obtaining dietary data, many investigators have used simpler but less reliable methods of dietary assessment, such as food frequency questionnaires and single 24 hour recalls, and finally, objective measures of diet are rarely, if ever, used in epidemiological studies.

The subjective methods are fraught with methodological problems including recall bias, and under-reporting of certain types of food and of total calories consumed.[107]

Given these limitations, it is not surprising that several earlier cohort studies failed to support an association between dietary fat, saturated fat and dietary cholesterol, and the risk of CAD[108,97] whereas others have revealed a significant association between dietary fat and serum cholesterol and the risk of CAD.[109,110]

2.1.4.iii Antioxidants: the difficulties associated with implementing substantial dietary change and the consistent biologically plausible finding in cohort studies, of the cardio-protective effect of substantial intakes of vitamin E and other antioxidant nutrients,[111,112] have led to several randomised controlled trials of dietary supplements. One trial of supplemental vitamin E demonstrated a reduction of cardiovascular events but not total mortality in the context of secondary prevention.[113] However, larger and more definitive studies involving vitamin E as well as β-carotene showed no protective effects.[114] There are a number of explanations for these apparently conflicting findings. These include the possibility that interventions might need to start early or continue for a longer duration or that the dose or form of the antioxidant supplement needs to be appropriate or physiological, or that a blend of antioxidant nutrients such as occurs in food might be more appropriate than mega-doses of a single antioxidant nutrient. More recently, it has been suggested that vitamin E might exert a cardiovascular protective effect by modulation of the inflammatory response.[115] Inflammatory biomarkers have recently been shown to be important predictors of CHD.[116] However, very high doses of vitamin E appear to be necessary to reduce the inflammatory response and no relevant clinical trial data are available.

2.1.4.iv Fibre: cohort studies among men have identified an inverse association between fibre intake and myocardial infarction[117] and have documented that a greater intake of foods rich in fibre significantly reduced the risk of IHD.[118] The intake of complex carbohydrates[97] and consumption of vegetables and fruits[119] have also been inversely associated with the risk of CAD. There are several mechanisms through which fibre protects against coronary disease. Water soluble fibre has a cholesterol lowering effect, but this appears to be relatively modest and does not account for the substantial effect of fibre on CAD incidence.[120] Other possible mechanisms include reduction in blood pressure, better glycaemic control and reduced triglyceride levels.[121]

2.1.4.v n-3 fatty acids: the n-3 group of fatty acids, which include n-3 fatty acids from fish oils (eicosapentaenoate and docosahexaenoate acid) and plant oils (α linolenic acid) are potential antiatherogenic nutrients. In initial reports, Innuits who consume large quantities of fatty fish, were reported to have decreased serum concentrations of cholesterol, triacylglycerols, chylomicrons, and LDL; subsequent studies in Europe and the US and recent epidemiological studies and interventional trials have also confirmed this. The high consumption of oily fish has been repeatedly shown to be associated with reduced risk of cardiovascular disease in cohort studies and in one secondary prevention trial, in which twice weekly consumption was associated with a 29% reduction in mortality.[122] Plant

sources of n-3 fatty acids were incorporated into the margarines provided in the active intervention group in the Lyons Heart Study and it was suggested by the authors that these could have accounted, to a considerable extent, for the beneficial results. However, in terms of effects on lipoprotein metabolism, the effects of n-3 fatty acids derived from fish are far superior.[123] The n-3 fatty acids may reduce cardiovascular risk by reducing the inflammatory response, by reducing platelet aggregation and blood pressure, reducing triglycerides, and by their anti-dysrhythmic effect. [124,125]

2.1.4.vi Trans fatty acids: intake of foods that are major sources of the trans isomer of fatty acids, formed from the partial hydrogenation of vegetable oil to produce margarine and vegetable shortening is known to be significantly associated with occurrence of CHD.[126] The association of trans fatty acids with increased risk of CHD was reviewed in 1995, and cohort studies provided evidence that excess intake of trans fatty acids can adversely affect cholesterol profiles and lead to increases in the risk of developing CHD.[127]

2.1.5 Diabetes

Diabetes is a major risk factor for CVD and is designated a CAD risk equivalent in ATP 111. The increased risk may be the result of many factors, such as plasma lipid abnormalities (high triglyceride, low HDL, sometimes high cholesterol and small dense LDL); the accompanying hypertension, nephropathy, obesity, insulin resistance and hyperglycaemia itself. Lipid abnormalities are usually more frequent in untreated non-insulin dependent diabetes and glucose intolerance than in treated type 2 diabetes and are improved by glycaemic control, especially if body weight is normal. There is a greater risk of diabetes in women than in men, and therefore, the usual sex difference in coronary risk in narrowed in diabetics. Compared with non-diabetics, the relative risk of CVD for men with diabetes is two to three, and that for women with diabetes is three to four.[128,129]

In an epidemiological analysis of the UKPDS results, a continuous relationship between glucose levels and cardiovascular events was observed. For every 1% decrease in haemoglobin A1c, the risk of all-cause mortality, myocardial infarction and stroke decreased 17%, 18% and 15%, respectively (p-value=<0.0001 for all three).[130] The UKPDS clearly showed that a policy of intensive glycaemic control for patients with type 2 diabetes has many positive health benefits that are likely to include cardiovascular benefits.

In addition to increasing the risk of CVD, diabetes also confers a worse prognosis after a cardiovascular event. Data from large prospective studies show that the relative risk of mortality following a myocardial infarction is two to three times higher in diabetic than in non-diabetic individuals.[131] In a recent study, the association between total homocysteine (tHcy) and cardiovascular disease was shown to be stronger in diabetic than in non-diabetic subjects[132] and atherogenic changes in the pre-diabetic state were mainly reported in insulin-resistant subjects.[133]

2.1.6 The insulin resistance or the metabolic syndrome

The insulin resistance or the metabolic syndrome represents a constellation of lipid and non-lipid related risk factors of metabolic origin; the underlying abnormality is the impairment of the ability of insulin to stimulate glucose uptake; defects of insulin action manifest as abdominal obesity and fasting hyperinsulinaemia. The compensatory hyperinsulinaemia necessary to maintain glucose tolerance in insulin resistant individuals is frequently associated with a cluster of metabolic abnormalities seen in individuals with central obesity and is characterized by glucose intolerance, dyslipidaemia and hypertension. Insulin resistance can also be associated with hyperinsulinaemia, hypertriglyceridaemia, low HDL, small dense LDL, hyperuricaemia, and elevated PAI 1. Recently, a genetic basis for this syndrome has been hypothesized[134] and chronic sub-clinical inflammation has been reported as being part of this syndrome.[135] Increased body fat (particularly abdominal obesity) and physical inactivity promote the development of insulin resistance; some individuals are also genetically predisposed to it. According to the ATP 111 guidelines, the diagnosis of the syndrome is made when three or more of the following risk determinants are present: waist circumference more than 102 cm in males and 88 cm in females; triglycerides >150 mg/dl; HDL <40 in men and <50 in women; blood pressure >130/85 mm Hg and fasting blood glucose >110 mg/dl. From a therapeutic standpoint, the treatment of this condition should not only aim at reducing plasma triglyceride levels, but also at improving all features of the insulin resistance syndrome, for which body weight loss and mobilization of abdominal fat appear as key elements. Cardiovascular risk factors associated with the metabolic syndrome are likely to significantly contribute to early onset diabetes.[136]

2.1.7 Obesity

Obese adults experience higher rates of cardiovascular disease.[137] Obesity is often present in association with other risk factors for CAD and this grouping further increases the risk. In most studies, body fat has been used to estimate overall obesity. Such indices fail to discriminate body fat from muscular and skeletal mass; moreover it is the site of fat accumulation, which is considered a predominant factor for metabolic disorders of obesity. Abdominal obesity or central adiposity, which means the preferential deposition of adipose tissue around the trunk and intra abdominally, a pattern of obesity as measured by waist circumference has emerged as an even more important risk factor for CAD and diabetes than total body adiposity,[138] and is more closely associated with the cardiovascular disease risk factors studied than overall adiposity as measured by BMI.[139,140] Increased abdominal fat is also associated with insulin resistance,[141,142] although the mechanism linking the two have not been fully elucidated. It has been suggested that abdominal visceral fat cells may be resistant to the antilipolytic effect of insulin,[143] resulting in increased hepatic availability of NEFA; the latter increases gluconeogenesis, reduces hepatic insulin extraction, and stimulates VLDL apolipoprotein B and triglyceride production.[144] Increased NEFA are also associated with reduced insulin mediated glucose uptake leading to glucose intolerance and hyperinsulinaemia.

2.1.8 Physical activity

Several studies have demonstrated that a higher level of CVD mortality accompanies sedentary lifestyle and low level of cardio-respiratory fitness.[145] Enhanced levels of cardio-respiratory fitness confer resistance to elevations in CAD risk factors.[146] Mechanisms that may account for the influences of regular physical activity on the proneness to CVD include attenuation of other common risk factors, antithrombotic effects, increased myocardial vascularity and function and improved metabolic environment.[147] The benefits of physical activity are also known to extend to those with multiple risk factors.[148] The initiation of physical activity therefore, has practical implications in the primary and the secondary prevention setting.[149] Current recommendations advise people of all ages to include a minimum of 30 minutes of physical activity of moderate intensity on most days, and preferably all days of the week. Accumulation of shorter sessions of physical activity as opposed to requiring one longer, continuous session of exercise is also known to be beneficial.[150]

2.1.9 Family history

CAD is affected both by environmental as well as genetic factors; family history is a strong risk factor for CAD and its effect is synergistic with other cardiovascular risk factors.[151] Genes contribute to CAD development and progression, and response to risk factor modification and lifestyle choices. Variation in factors such as LDL, apolipoprotein E, HDL, apolipoprotein A-I/CIII/A-IV, lipoprotein lipase, cholesterol ester transfer protein, lipoprotein (a), and homocysteine may affect CAD risk via genetic or environmental mechanisms or their interactions. Understanding the genetic basis of CAD is important as it has implications for management and prevention.

Many of the inherited cardiovascular risk factors can be modified, such as LDL cholesterol, homocysteine and lipoprotein (a). Early detection of CAD might lead to earlier intervention for genetically susceptible individuals. However, data are lacking regarding the efficacy of this approach in preventing CAD clinical events. Despite this lack of evidence, knowledge of genetic CAD susceptibility has value in providing risk related information and guiding decision-making. Further research that investigates outcomes regarding genetic risk assessment for CAD is necessary.[152]

2.1.10 Psychosocial factors

Among the variety of psychosocial factors that have been studied, the evidence is clearly stronger for some than for others. Although early research implicated type A personality in the development of CAD, subsequent studies did not support the association.[153] Recent studies provide clear and convincing evidence that psychosocial factors contribute significantly to the pathogenesis and expression of CHD; this evidence is composed largely of data relating CAD risk to depression, anxiety, personality factors and character traits, social isolation, and chronic life stress.[154] Patho-physiological mechanisms underlying the relationship between these entities and CAD can be divided into behavioural mechanisms,

whereby psychosocial conditions contribute to a higher frequency of adverse health behaviours.[155]

Emotionally stressed younger patients are known to represent high-risk groups;[156] in a recent study, the association between trait anger and the risk of CAD was examined prospectively, results revealed that proneness to anger placed normotensive middle aged men and women at a significant risk of CAD morbidity and death independent of established biological risk factors.[157] The evidence is also convincing for the risk associated with depression;[158,159] recent studies have shown that depressive symptoms constitute an independent risk factor for the development of CHD and total mortality.[160] This evidence, although strong, is indirect; for example, even though depression is an important predictor of mortality after myocardial infarction,[161] it is premature to conclude that the relationship is causal. Although less strong than the evidence for depression, data also support risks associated with anxiety, particularly phobic anxiety, and psychological distress, as well as low social support. The degree of risk associated with depression, and to a somewhat lesser degree with anxiety and low social support, is as great as that associated with more traditional risk factors and is largely independent of them. For example, after control for disease severity, measures of depression or hopelessness have been associated with relative risks between 1.5 and 2 for fatal CHD or myocardial infarction over periods ranging from 6 to 27 years in community samples of initially healthy individuals.[158,159] Data also indicates that depression (and to a lesser extent anxiety) and low social support can influence behaviourally alterable cardiac risks such as smoking, exercise and diet, as well as patients' tendencies to comply with recommended medications or to respond to symptoms by seeking treatment rapidly.[162]

Biologically plausible mechanisms of action for psychosocial factors have suggested[163,164] that depression is associated with chronic disturbances in autonomic balance and platelet aggregation as well as chronic increases in neuroadrenergic tone, which lower arrhythmic threshold. Transitory emotional states such as anxiety and anger can also provoke sudden changes in heart rate, peripheral vascular tone and probably coronary vascular tone,[165] which can induce ischemia and lower the arrhythmic threshold. In addition, the release of adrenaline that accompanies fear or anxiety can stimulate platelet aggregation via alpha-adrenergic pathways.

2.1.11 Socio-economic factors and social support

Several studies have confirmed a graded, inverse relation between socioeconomic stress (SES) and the risk of CAD, CVD and all-cause mortality.[166,167] This disparity in cardiovascular health between people of low and high SES seems to be on the rise;[168] among British men, between the early 1970's and early 1980's, CAD mortality rates declined by 15% in men in non-manual occupations and increased by 1% in those in manual occupations.[169] In the Whitehall Study, the social class difference was only partly explained by adjustment for known coronary risk factors. Other studies however have yielded somewhat inconsistent effects of SES.[170,171]

Evidence also suggests that low SES in childhood may have an independent effect on risk of CAD in adulthood (relative risk of 1.3 to 1.9);[172,173] however, some studies have found no relationship.[174,175] Childhood poverty followed by high standards of living is known to operate, at least partly, as a risk factor for CAD through conventional risk factors.[176] Lower SES in childhood is associated with higher levels of hostility, depression and hopelessness, greater tobacco consumption and alcohol abuse, less leisure time physical activity, obesity and a less nutritious diet in adulthood.[177]

There is also an inverse relationship between SES and conventional risk factors.[178] Concurrence of risk factors that can have a synergistic effect on the risk for CVD has been shown to be higher in the less educated groups.[179] Risk factors contribute to, but do not fully explain, health inequalities among SES groups and adjustment for these attenuates but does not eliminate the relationship between SES and cardiovascular mortality.[180] This suggests that there are other pathways involved in the mediation of socioeconomic inequalities in CAD risk. The indirect effect of psychosocial circumstances may include increased exposure to behavioural risks resulting from psychosocial stress (such as stress-related smoking, drinking or eating for comfort). The direct effects of low SES are likely to centre on the physiological effects of chronic mental and emotional stress.[181] Chronic stresses associated with social position may also modify neuroendocrine and physiological functioning.

2.1.12 Physical characteristics

Barker et al, showed for the first time in 1990 that intrauterine environment had an important effect on blood pressure and diabetes in adults.[182] Low birth weight, thinness and short body length at birth are known to be associated with increased rates of cardiovascular disease and non-insulin dependent diabetes later in life.

Evidence that CAD, hypertension and diabetes are programmed came from the longitudinal study of 25,000 men and women in UK in which size at birth was related to the occurrence of disease in middle age. People who were small or disproportionate (thin or short) at birth had high rates of CAD, high blood pressure, high cholesterol concentrations and abnormal glucose metabolism.

According to the foetal origins hypothesis, these diseases originate through cardiovascular, endocrine and metabolic intrauterine adaptations secondary to in-utero programming in response to maternal malnutrition.[183] These relations were seen to be independent of the length of gestation, suggesting that cardiovascular disease is linked to foetal growth restriction rather than to premature birth.[184] “The foetal insulin hypothesis”, on the other hand, proposes that genetically determined insulin resistance results in impaired insulin-mediated growth in the foetus as well as insulin resistance in adult life. Low birth weight, measures of insulin resistance in life and ultimately glucose intolerance, diabetes and hypertension could then all be phenotypes of the same Insulin resistance genotype.[185] Although the influences that impair foetal development leading to and causing adult cardiovascular disease remain to be defined, there are strong pointers to

the importance of foetal adaptations invoked when materno-placental nutrient supply fails to match the foetal nutrient demand.

Recently low birth weight is reported to be associated with endothelial dysfunction in young adults; an effect most marked in individuals with lower risk factor profiles and may be relevant to the pathogenesis of atherosclerosis in later life in individuals with low levels of cardiovascular risk factors.[186]

2.1.13 Coagulation factors

The development of CAD and myocardial infarction has both, atheromatous and thrombotic components and evidence exists to implicate a number of coagulation and fibrinolytic proteins in this process. Case-control and prospective studies of myocardial infarction in Whites have reported elevated fibrinogen,[187] FVII C,[188] plasminogen activation inhibitor 1 (PAI 1),[189,190] tissue plasminogen activator (t-PA)[191] and activated FXII [192] as being related to outcome.

Fibrinogen has been identified as an independent risk factor for CVD. In their meta-analysis of six prospective observational studies totalling 92,147 person-years, Ernst and Resch[193] calculated an odds ratio for IHD of 2.3 (95% C.I. 1.9, 2.8) for elevated plasma levels of fibrinogen. Although there were some interactions between fibrinogen and other risk factors such as smoking, diabetes, different lipid fractions such as LDL-cholesterol and Lp(a) and menopause, fibrinogen was considered an independent factor in most studies and a strong marker in others.

Fibrinogen is also known to be associated with traditional risk factors.[194] This suggests that elevation of fibrinogen may provide a mechanism for risk factors to exert their effect and through this, fibrinogen may increase cardiovascular risk. It has been shown that increased risk of CVD associated with smoking[195] and obesity[196] may be mediated in part through fibrinogen. Fibrinogen binds to platelets via glycoprotein IIb, IIIa contributing to platelet aggregation, promotes fibrin formation and increases plasma viscosity. Fibrinogen also clusters with several components of the metabolic syndrome thus increasing cardiovascular risk; additionally, fibrinogen is also an acute phase reactant and is increased in inflammatory states.[197] This further highlights its potential role in mediating inflammatory mechanisms associated with cardiovascular risk.

Factor VIII and von Willebrand factor, which are bound together, have also been proposed as risk factors; this has been supported by some studies[198] whereas others have yielded inconsistent results.[199] In a recent cohort study, high-activated factor XII was associated with increased CHD risk, but low levels were not protective. Plasma VIIa and factor X activation peptide were independently and inversely related to risk. Plasma factor IX activation peptide and fibrinogen were positively associated with risk, but the relations were no longer statistically significant after adjustment for other factors, including VIIa and apoA-1; other haemostatic markers were not associated with CHD risk. In this study,

haemostatic status did not add significant predictive power to that provided by conventional risk factors.[200]

Fibrinolysis is regulated by several factors, in particular, the balance between tissue plasminogen activator (t-PA) promoting lytic action and PAI-1 inhibiting lyses; elevated PAI-1 inhibits t-PA release from vascular endothelial cells. t-PA antigen and PAI-1 are elevated when fibrinolysis is prolonged. Elevated PAI 1 has been documented in young patients following an acute myocardial infarction and has been associated, when elevated, with a higher risk of recurrent events;[201] this association does not persist in older patients. Further studies are required to establish whether these fibrinolytic components are independent risk factors. PAI-1 and t-PA antigen are strongly linked to the metabolic disorder of insulin resistance, implying a pathogenic role in vascular disease in subjects with insulin resistance and type 2 diabetes.[189]

Findings have suggested that certain factor VII genotypes have a role in protection against myocardial infarction, which may explain why some patients do not have myocardial infarction despite the presence of strong atherosclerosis.[202] Further research is warranted to clarify these issues.

2.1.14 Homocysteine

A meta-analysis of 14 prospective studies has calculated a pooled odds ratio for CAD for a 5 µmol increase in homocysteine of 1.20 (95% C.I. 1.14, 1.25).[203] Elevated levels of plasma total homocysteine can result from genetic or nutrient related disturbances in the transsulfuration or remethylation pathways for homocysteine metabolism.[204] The concept has been based on the observations of premature vascular disease in patients with homocystinuria, the relation between total homocysteine (tHcy) and both clinical CVD as well as atherosclerotic disease, the relation between tHcy in children and CVD in their parents or relatives[205] and reduction in CVD surrogate endpoints after tHcy lowering interventions with B vitamins.[206]

Several biologically plausible mechanisms have been suggested for occlusive vascular disease associated with hyperhomocysteinaemia. These involve the platelets, the coagulation system, the endothelium and the vessel wall[207] and promotion of leukocyte recruitment.[208] It has been suggested that the deleterious effect on the normal pro-thrombolytic and anticoagulant activities of endothelial cells occurs by perturbation of the vascular endothelial cell protein C mechanism.[209,210] This strongly suggests that elevated homocysteine levels are atherogenic by inducing a pro-coagulatory state.

Hyperhomocysteinaemia has also been shown to cause in vivo interference with nitric oxide dependent reactive vasodilatation,[211] which lends support to the idea that elevated homocysteine, provokes acute vascular events especially in patients with other risk factors. The fact that the response is observed in healthy subjects precludes confounding by other risk factors. This is further supported by the observation that acute

hyperhomocysteinaemia after methionine loading is associated with acute endotheliemia.[212]

In acute coronary syndromes, elevated homocysteine levels have a pro-thrombotic effect[213] and strongly predict late cardiac events.[214] Homocysteine is also a significant predictor of mortality in patients with angiographically defined CAD,[215] independent of traditional risk factors, CRP, and MTHFR genotype.[216] It is also known that even mild to moderately increased levels of homocysteine, irrespective of the cause, strongly increase the risk of atherosclerosis and that a graded risk of adverse events is found throughout the normal range of homocysteine levels.[217] This suggests that patients with normal homocysteine levels should also benefit from homocysteine lowering therapy.

Some observations have, however, raised questions about tHcy as a risk factor and a few prospective studies have shown a weak to no relation between tHcy and CVD. It has also been pointed out that several traditional risk factors are associated with tHcy and that this may confound the relationship; tHcy is also known to be related to renal function and it has been suggested that hyperhomocysteinaemia may reflect early nephrocalcinosis. The C677T transition of methylenetetrahydrofolate reductase gene, which causes the fairly common thermo labile variant of N5, N10 methylenetetrahydrofolate reductase and leads to an increase of about 50% in plasma homocysteine concentrations, also seems to confer no extra vulnerability in vascular disease.[218,219] An alternative interpretation is that moderate increases in homocysteine concentration are a marker of tissue damage or repair, and that the association between hyperhomocysteinaemia and vascular disease emerges only after the vascular event.[220] However, the strength of some of these arguments can be questioned because there is increasing evidence that increased tHCy is a proximate risk factor provoking the acute event, it strongly interacts with traditional risk factors and it may predict CVD death in patients with chronic renal failure. Furthermore, studies on the C677T polymorphism lack statistical power[221] and the TT gene may even modulate CVD risk independently of homocysteine. Thus, only placebo controlled intervention studies with tHCy lowering B vitamins and clinical endpoints can prove additional valid argument for the debate over whether tHCy is a causal CVD risk factor.[203]

A number of RCT's involving the use of fairly large doses of these nutrients as dietary supplements are underway. These are intended to establish whether tHcy is indeed a causally associated risk factor as well as the potential benefit of supplements. The results of the first of these have recently been published.[222] A combination of folic acid (1 mg), vitamin B12 (400 µg) and pyridoxine (10 mg) or placebo was administered to 206 patients following successful angioplasty in a prospective double blinded intervention trial. Treatment with vitamins significantly reduced homocysteine and decreased the rate of restenosis and the need for revascularisation. If these results are confirmed, supplementation with these vitamins will undoubtedly become routine treatment in the management of patients with cardiovascular disease and perhaps also in the context of primary prevention.

Indeed, given the widespread use of folic acid fortified foods in many westernised countries, it is conceivable that some of the decline in CHD is explained by a reduction in tHcy, a phenomenon clearly demonstrated to have occurred in the United States.[223]

Although cysteine is structurally similar and metabolically linked to tHcy, its relation to the risk of cardiovascular disease has received little attention. A recent study has shown a significant U-shaped relationship between total cysteine and cardiovascular disease after adjustment for tHcy, creatinine, and other cardiovascular disease risk factors.[224] This hypothesis needs to be further investigated.

2.1.15 Inflammation and infection

Atherosclerosis is known to be an inflammatory disease;[225] the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses.[226] Several pathophysiologic observations in humans have led to the formulation of the "response to injury hypothesis" of atherosclerosis, according to which, technically, endothelial denudation and functionally, endothelial dysfunction is the first step in atherosclerosis. Possible causes of endothelial dysfunction may be elevated and modified (oxidized) LDL, free radicals caused by smoking and hypertension (through muscle overgrowth), diabetes, elevated levels of homocysteine, genetic alterations, infectious organisms and a combination of these factors. The adhesion and transendothelial migration of leucocytes is thought to be important in the initiation and progression of atherosclerotic disease. These processes are known to be initiated by cell adhesion molecules (CAMs).[116]

Independent associations between most of the markers of inflammation and chronic CAD have been demonstrated in clinically stable patients with CAD.[227] Several large scale prospective epidemiological and clinical studies have shown that plasma levels of C-reactive protein (CRP), which is an acute phase reactant and an extremely sensitive marker of systemic inflammation, is a strong independent predictor of the risk of future myocardial infarction, stroke, peripheral arterial disease and vascular death amongst individuals without known CVD.[228,229] In addition, in patients with known myocardial infarction, stable angina pectoris and acute coronary ischaemia, levels of CRP are associated with increased vascular events.[230,231] CRP, therefore has the potential to play an important role as an adjunct for risk assessment.[232] It is not clear however, whether increased CRP reflects the extent and severity of atherosclerotic lesions or contributes to atherothrombosis itself. Evidence for the latter comes from the observation that CRP has been shown to selectively bind to modified LDL within inflammatory plaques; statins have also been shown to decrease both CRP and LDL.[233,234] Cell adhesion molecules, which is another inflammatory biomarker is known to be associated with an increased risk of future vascular events[228] and incident CAD; however, soluble CAMs are of limited prognostic value.

Recent studies have also demonstrated that inflammation and infection causes HDL to lose its anti-inflammatory properties.[235] Recent reports of increased levels of TNF-alpha in patients with myocardial infarction further support the hypothesis that, persistent

inflammatory instability is present among stable patients at increased vascular risk suggesting the possible role of novel therapies to attenuate the inflammatory response in the treatment of myocardial infarction.[236]

Whereas there is no doubt that atherosclerosis is an inflammatory process, its initiation has been the subject of debate over the last decade. Several studies have addressed the possible role of infectious agents in the pathogenesis of atherosclerosis and CAD as a result of which, many infectious agents have been implicated in the pathogenesis of CAD. Recently infectious burden has been correlated with CHD[237,238] and the simultaneous presence of antibodies has been shown to substantially increase the risk for disease development.[239] Interest in the concept has been rekindled because of technological advances in tissue localization of various proteins; in addition, the ability to amplify specific DNA sequences in virtually any tissue specimen has opened novel avenues of investigation. These infectious agents include adenovirus, entrovirus, cytomegalovirus and herpes simplex virus as well as chlamydia pneumoniae (Cpn) and helicobacterium pylori.

Evidence for chlamydia pneumoniae as a potential causative agent in coronary disease,[240] stroke[241] and carotid[242,243] and femoral[244] atherosclerosis is strong and based on findings from several seroepidemiological studies and recently, pilot anti-chlamydial antibody trials. Initial trials involved treatment of subjects with established CAD with antibiotics directed against Cpn and reported decrease in events in the antibiotic treatment group[245,246] and improvement in global tests of markers of inflammation;[247] however later, absence of marked early reductions in ischemic events were reported.[248] On the other hand, helicobacterium pylori is a controversial issue; further studies may be needed to confirm or refute the existence of any modest associations.[249,250]

CMV seropositivity and elevated CRP, especially when in combination, are strong, independent predictors of mortality in patients with CAD, suggesting that a chronic, smouldering infection might have the capacity to accelerate the atherothrombotic process.[251] Recently CMV seropositivity in patients with an inflammatory response is shown to be independently associated with future cardiac mortality, whereas this association is lost in patients who do not demonstrate an inflammatory response. These data support the hypothesis that the atherosclerotic effects of CMV are mediated through an underlying inflammatory response.[252]

Thus there is sufficient evidence to suggest that inflammatory mechanisms play a part in mediating coronary risk. However the specific initiating event has been the subject of debate. In addition to infection, an autoimmune role for CAD has also been hypothesized;[253,254] inflammation has also been linked to the insulin resistance syndrome,[135] thus expanding the list of possible initiating agents and events.

2.1.16 Hormone replacement therapy

Gender differences in cardiac function and metabolism are attracting considerable interest, particularly in relation to mechanisms responsible for the relative resistance of pre-menopausal females to CAD. The delay in onset of cardiovascular disease in women before the menopausal years suggests that female sex hormones might possess cardio-protective properties.

Based on the existing body of evidence, the role of hormone replacement therapy (HRT) in the female patient is contentious. Population based observational studies of both oestrogen/progestin use in women, not known to have CHD and meta-analysis of these studies, almost uniformly suggests a 35-50% reduction in CHD risk,[255] but it is also possible that these women are better educated, more compliant and have healthier lifestyles. However the Heart and Estrogen/Progestin Replacement Study (HERS),[256] has radically altered this view. In this clinical trial, postmenopausal women less than 80 years of age who received oestrogen plus progestin in the secondary prevention setting did not experience a reduction in overall risk of nonfatal myocardial infarction and CHD death or of other cardiovascular outcomes. Other randomised trials of HRT[257] and emerging data from HERS are likely to answer some of the questions raised by HERS to determine the effectiveness of HRT in the secondary prevention setting. Example of an intervention failing in secondary prevention but succeeding in primary prevention in unknown; therefore, the use of ERT in the primary prevention setting is also, likely to remain contentious.[258]

2.1.17 Trace elements

Iron is known to play a catalytic role in the oxidation of LDL plaques.[259,260] Excess iron has been proposed to be a risk factor in the development of acute MI in several[261,262] but not all,[263,264] prospective human studies. This discrepancy may be due to measurement variability in the methods used for accessing body iron stores as serum iron, transferrin saturation and serum ferratin measurements are very responsive to inflammation and various disease processes and have large biological and analytical variability.[265] Other studies such as the ratio of transferrin receptor to serum ferratin (TfR/ ferratin) may be a better estimation of body iron stores.[266] Because of all these considerations, published prospective studies do not provide good evidence to support the existence of a strong epidemiological association between iron status and CAD.

2.1.18 Alcohol

A consistent coronary protective effect has been observed for consumption of 1 to 2 drinks per day of an alcohol-containing beverage; however, higher intakes are associated with increased total mortality.[267] Evidence from cohort studies confirms that moderate intake of alcohol (not more than two drinks per day) is inversely associated with the risk of developing IHD;[268] the risk reduction being the same in diabetics and non-diabetics.[269]

The reduced risk of CHD associated with alcohol is believed to be due, at least in part to the effect of alcohol on the lipoprotein profile, including the favourable effects on HDL and its sub-fractions.[270,271] In the case of wine consumption, the protective effect of wine is ascribed to the potent antioxidant effect of flavanoids. Flavonoids and whole juice from purple grapes is known to inhibit platelet function and enhance nitric oxide release; the suppression of platelet-mediated thrombosis represents a potential mechanism for the beneficial effects of purple grape products, independent of alcohol consumption, in cardiovascular disease.[272] Red wine is also known to prevent activation of mononuclear cells.[273]

2.1.19 Summary

Several biological, behavioural, psychological and social risk factors have been recognized as risk factors for CAD. Several aspects need to be recognized in this context. Firstly, that the risk for most biological variables operates on a continuum of progressive increase over a wide range, rather than across abrupt arbitrary thresholds, which spells out the need to address the risk factor distribution in a population rather than deal only with those at the extreme high end. Secondly, multiplicative risk arising from a combination of risk factors has also been recognized from several large longitudinal studies; it is now clear that the largest proportion of CVD events in any community arises from persons who have modest elevations of many risk factors than from individuals with marked elevation of a single risk factor. And thirdly, clustering of CVD risk factors is also common due to similar life-style associations, which provides an opportunity to adopt integrated strategies of multi-factorial risk reduction.

Several differences in risk factor occurrence and association occur between South Asians and the developed world populations, it is important to highlight these differences as they have important implications for risk reduction strategies. As insight into the risk profile of Pakistanis came from the study of expatriate South Asians living in the developed countries, the subsequent section of the literature review will focus on expatriate South Asian coronary risk before discussing the epidemiology of risk factors in the indigenous Pakistani population.

2.2 EPIDEMIOLOGY OF CORONARY ARTERY DISEASE IN SOUTH ASIAN IMMIGRANTS

2.2.1 CAD in South Asian immigrants

The highest prevalence (mortality and morbidity rates) of coronary heart disease in any ethnic group has been noted in immigrant South Asians (Indians, Pakistanis and Bangladeshis) living in Western Europe and North America.[16,17] This phenomenon was initially described in colonial plantation workers in the 1950's[274] whilst it was first recorded in the UK in 1971.[275] In England and Wales in 1970-72, mortality from CAD was

20% higher in men and women who had been born in South Asia than in the general population.[276] Studies on Indian emigrants to the USA[277] have also confirmed high prevalence and mortality rates. Mortality data for South Asians from other parts of the world is summarized in Table 2.2 Where South Asians are compared with populations at high risk of CAD, the relative risk is comparatively lower, as opposed to the comparison with other groups at relatively low risk for CAD, where the relative risk associated with South Asian origin is much higher.

Table 2.2 Mortality data for immigrant South Asians

Country	Years	Groups contrasted	Age	CAD mortality
Singapore[278]	1980-84	S Asian/Chinese	30-69	3.8 (males), 3.4 (females)
	1980-84	S Asians/Malays	30-69	1.9 (males), 1.6 (females)
Fiji[279]	1980	S Asian/Melanesian	40-59	2.4
Trinidad[280]	1977-86	S Asian/African	35-69	2.1 (males)
South Africa[281]	1985	S Asian/European	35-74	1.4
England[282]	1979-83	S Asian/European	20-69	1.4

In a prospective study of men in Trinidad, the incidence of CAD per 1000 person years was found to be 16.4 in men of Indian descent, 6.8 in men of African descent and 6.2 in those of European descent.[283] In another survey carried out in East London, mortality and CAD attack rate were higher in South Asians compared with the rest of the population in the East London Borough.[284] In the South Hall study,[18] comparing 1421 South Asians and 1515 European men in the age group of 40-69 years, prevalence of ECG abnormalities was higher in South Asian compared with Europeans (17% vs. 12%, p-value= <0.001) with an excess of major Q waves (Minnesota codes 1-1 or 1-2) in younger South Asian men (p-value=0.001 for the age-ethnicity interaction); ECG abnormalities have also been shown to be higher amongst South Asians compared with Europeans (6% vs. 2% in men) in another recent study.[285] Similarly, data from Canada also shows that being of South Asian ethnic origin carries a 4.51 higher odds of CAD compared with Europeans after adjusting for both conventional as well as novel risk factors.[286]

Recently, a longitudinal study has confirmed the increased susceptibility of Asian Indian males to CAD; in 24,968 person years of follow up, Asian Indians were found to be at greatest risk of CHD, compared with Chinese (Hazard Ratio 3.0, 95% C.I. 2.0, 4.8) and Malays (HR 3.4, 95% CI 1.9-3.3).[287]

2.2.2 CAD in native South Asians

Reviews of prevalence studies from India have shown a two fold increase in prevalence in rural areas (from 2.96% to 4.14%) and a nine fold increase in prevalence in the urban areas (from 1.04% to 9.45%) over the last three decades.[288] South Asian countries with a sizable population such as Pakistan and Bangladesh have poor prevalence data on CAD.[289] Cross sectional general health and population surveys do however, show high prevalence of conventional risk factors.[14] However, causal and temporal relationships between risk factors and CAD prevalence are poorly defined in native South Asian

populations. In this context, South Asia has also been conspicuously absent from several global initiatives established to track CAD risk.[290]

2.2.3 Natural history of CAD in immigrant South Asians

In addition to high prevalence, CAD in South Asians is known to have a significantly younger onset and is known to be more aggressive in its presentation.[291,292] Angiographic data from Malaysia, where South Asians comprise 10% of the population, reveals that 56% of patients with CAD below 40 years of age are South Asians, implying a 10-15 fold higher rate of CAD compared with the native population.[293] Similarly, facility-based data from Qatar reviewed cases with documented myocardial infarction; 23% of these cases were less than 40 years of age; amongst these, 42% were Asian in origin although Asians are a minority population in Qatar.[294] The standardized mortality ratio (SMR) for CAD among Asian Indian males in UK is twice the national average between the ages of 30 and 39 and more than three times the national average between the ages of 20-29.[295] Thus the higher overall risk in South Asians is even more exaggerated in the younger age group.

Narrowing of sex difference in coronary risk amongst South Asians has been reported the world over.[296] Some of the immunity of women from CAD seems to be lost in South Asian populations.[297] The SMR for CAD among Asian Indian immigrant women in UK is 1.5 times higher than of native British (Caucasian) women and 2.6 times higher than that of the other immigrant women.[295] Asian Indian women in South Africa also have the highest SMR for CAD of all ethnic groups i.e. 4 times higher than that of US women, 14 times higher than that of French women and 21 times higher than that of Japanese women.[281] The adverse cardiovascular risk in migrant South Asian women is explained to some extent by their anthropometric indicators, which are dependent on lifestyle factors and parity.[298]

Data from India and UK has shown that compared with Caucasians, South Asians also have a far more malignant pattern of CAD as evidenced by larger infarct sizes and adverse prognosis.[299] Facility based data from Birmingham has also shown that South Asians have a substantially higher frequency of triple vessel CAD (54 vs. 21%) compared with Caucasians.[300] The presentation and natural course of first myocardial infarction in migrant South Asian and indigenous populations in Britain was studied in 77 white and 54 Asians presenting consecutively with the first myocardial infarction; overall, the relative rate of infarction was 4.9 times higher in South Asians (95% C.I. 3.4, 6.9) than in the White population; mean age of infarction was also lower in Asians (50.2 years) compared with the White population (55.5 years). Additionally, in South Asians, infarct size (as assessed by CPK, radio nucleotide ventriculography and contrast ventriculography), the extent of coronary atheroma and the mean number of plaques were higher and a higher proportion of South Asians had triple vessel disease (p-value=<0.001).[301] South Asians have also been reported to have smaller coronary arteries with important therapeutic implications.[302]

Studies from India and Pakistan have also shown a higher frequency of triple vessel disease, more diffuse coronary atheroma, poor survival after the first myocardial infarction, higher risk of recurrent myocardial ischaemia after myocardial infarction,[303,304] more frequent involvement of the left anterior descending artery[305] and multiple lesions in non infarct related arteries.[306]

2.2.4 Risk factors

South Asians, both indigenous and immigrant comprise a diverse group with a high risk of CVD. This excess CHD risk cannot be explained entirely in each South Asian sub group by conventional risk factors alone.307 This has led to several lines of investigation over the last two decades to identify factors that are responsible for this increased risk. Data reveals that expatriate South Asians do however, suffer from increased risk of type 2 diabetes,308 together with adverse fat distribution, hyperinsulinaemia and insulin resistance relative to other ethnic groups.309 This, known as the insulin resistance syndrome, is known to underlie the increased coronary risk in South Asian populations.18

2.2.4.i Smoking: prevalence of smoking in South Asians in Britain ranges from 57% in Bangladeshi men, 30% in Pakistani and Indian men (a figure comparable to the national average) to being very low in South Asian females and Sikhs.284 This disparity in smoking rates, while they share coronary mortality and prevalence rates, shows that smoking cannot be described as a major risk factor for the increased prevalence of CAD in South Asians overseas. The prevalence of smokeless chewable tobacco on the other hand, is very high in native South Asia, but its prevalence in the expatriate population has not been documented. The cardiovascular effects of chewable tobacco are similar to those of cigarette smoking.36 However, the age adjusted relative risk of dying from CVD is lower with smokeless tobacco than with tobacco smoking.37

2.2.4.ii Hypertension: average blood pressure levels vary significantly amongst Hindus, Sikhs and Muslims residing in UK, with Bangladeshis having lower blood pressure levels, the Gujrati Hindus and Muslims having similar blood pressure levels to the native population while the Punjabi Hindus and Sikhs have higher blood pressure levels.284,296 Comparative studies in UK have also shown that blood pressure levels in South Asians are not higher than in the Europeans,310 therefore the increased coronary prevalence and mortality rates cannot be attributed to elevated blood pressure alone.

2.2.4.iii Dyslipidaemia: mean serum total cholesterol level in South Asians in England is known to be lower than that of Whites;310,311 this has also been demonstrated for Pakistani migrants in particular.312 The results of a cross sectional analysis in Vellore, India, studying 1066 patients who presented for coronary angiography also revealed that CAD occurs in Indians at low lipid levels.313 On the other hand, the combination of low plasma levels of HDL and high levels of triglycerides, known to be synergistic predictors of CAD risk in prospective studies, is commoner in South Asian immigrants in association with type 2 diabetes.314,315 The greater atherogenicity of lipids in South Asians, despite relatively lower LDL levels could be explained on the basis of the chemical composition of

LDL, which, in such cases, is protein rich and cholesterol depleted, with a shift in the spectrum of LDL towards smaller and denser LDL.59 Asian Indians are known to have a higher prevalence of small, dense LDL compared with the White population.316,317 This is further reinforced by studies that have also demonstrated that diabetic Indians have a combination of low HDL and higher concentrations of triglycerides, apolipoprotein B and smaller LDL particles.318

2.2.4.iv Lipoprotein (a): South Asians are known to have higher levels of Lp(a) compared with North Americans319,320 and British White populations.321 Median Lp(a) concentrations have been found to be significantly higher in South Asians compared with Northern Americans in different studies (15 versus 11 mg/dl,322 16.9 versus 9.1 mg/dl323 and 28.4 versus 11.3 mg/dl286). In these studies, the percentage of Lp(a) values greater than 30 mg/dl have been reported in 25% South Asians compared with 19% White Americans322 and in 50% South Asians compared with 24% Canadians.286 Lp(a) levels in Asian Indian newborns have also been found to be significantly higher than in the Chinese in Singapore, in this study; the differences in Lp(a) levels in cord blood parallel the four-fold differences in adult CAD mortality between these two populations in Singapore.324

However several things relating to Lp(a) in the South Asian context need to be taken into account. Firstly, that elevated plasma Lp(a) confers genetic predisposition to CHD in South Asians and that nutritional and environmental factors further increase this risk. Secondly, Lp(a) is generally thought to be a risk factor for CAD when cholesterol concentrations are as high as in the British European populations; its atherogenicity is reduced in association with reductions in LDL cholesterol levels. In indigenous South Asians, levels of Lp(a) are high but as the LDL levels are low, the incidence of CHD is low. South Asians, on the other hand, acquire higher LDL cholesterol concentrations compared with the native White population on migration, with the trend towards the acquisition of westernised behaviours, consistent with a sedentary lifestyle, high salt and fat and low fibre diet and stresses in the new place of residence; therefore the increases in serum cholesterol after migration unmasks the underlying genetic risk of CAD conferred by high serum Lp(a).[320,321] Thirdly, CAD risk in patients with high Lp(a) is much greater in patients with low HDL than with high LDL levels;[93] HDL levels are known to be lower in South Asian individuals, which further increases the risk associated with higher Lp(a) levels. And lastly it has been shown for the Japanese and the Spanish populations with type 2 diabetes that Lp(a) levels are an independent predictor of future atherosclerotic events. [325,326] This observation is important, as South Asians are known to have both increased rates of diabetes as well as increased levels of Lp(a).

2.2.4.v Diet: high coronary risk in expatriate populations is not explained by any unfavourable characteristics of the South Asian diet.[327] In a survey of Asians in the London Boroughs of Brent and Harrow, in comparison with the British population, Asians were found to consume less saturated fat (S), cholesterol and more polyunsaturated fat (P) and vegetable fibre; the P/S ratio of the Asian diet was 0.85 compared with 0.28 for the British population; this was reflected in the very high linolenic acids in their plasma lipids. The plasma total cholesterol and HDL were similar to that of a British comparison

group.[328] Variation in the intake of protective n-3 fatty acids derived mainly from fish, which is lower in the vegetarian Hindus and high in the Bangladeshi population also does not seem to be the culprit.[296]

Earlier it was suggested that cholesterol oxides in *ghee*, which is commonly used for cooking in the subcontinent are responsible for the high rates of CAD in South Asians;[329,330] cross sectional surveys in India also showed that *ghee* (clarified butter) and vegetable *ghee* (trans fatty acids) have an association with CAD.[331] This, however, was not supported by later studies.[18] Moreover, it was established that *ghee* consumption amongst South Asian migrants in Britain was almost exclusively confined to the lower socioeconomic groups.[332] However, reduction of dietary saturated fat in the South Asian diet in general, and the partial replacement of unsaturated fat has been shown to bring about favourable changes in the total cholesterol, HDL and LDL cholesterol and is associated with lower cardiovascular risk.[333]

A recent study in Singapore examined the possible roles of antioxidants and pro-oxidants in the differential risk of CHD between Indians, Malays and Chinese. It was suggested that lower vitamin C and selenium in Indians, particularly in combination, could play a part in their increased risk of CAD; lower vitamin C was thought to be a result of destruction due to prolonged cooking, which is a common practice in South Asian households.[334]

2.2.4.vi Diabetes and the insulin resistance syndrome: diabetes in association with affluence and obesity was described in ancient Indian medical treatises 2000 years ago.[335] As with the high rates of CAD, the high rates of diabetes in South Asians in UK are common with that overseas elsewhere. Prevalence surveys based on WHO criteria are now available from areas where South Asians are in large numbers. In these surveys, the prevalence of diabetes in men and women above the age of 40 is 20% compared with 5% in other ethnic groups. [336,337] Recently in a survey carried out in UK, the age standardized (35-79 years) prevalence of diabetes in a Pakistani population was reported at 33% compared with 20% in Europeans and 22% in Afro Caribbeans.[308] However, the overall quantitative consideration of the relationship between glucose intolerance and CAD mortality suggests that the markedly elevated CAD risk for an entire population is not easily explained by the high prevalence of glucose intolerance alone.[338,339] American Pima Indians, and Polynesian Nauruan Islanders have high prevalence of diabetes but a low incidence of nonfatal and fatal CAD.[340]

Data reveals that South Asians do however, suffer from increased risk of non-insulin dependent diabetes mellitus together with adverse fat distribution, hyperinsulinaemia and insulin resistance relative to other ethnic groups.[309,341] This, known as the insulin resistance or the metabolic syndrome is known to underlie the increased coronary risk in South Asian populations;[18] the syndrome represents a constellation of lipid and non lipid related risk factors of metabolic origin, the hallmark abnormalities of which are, central obesity, glucose intolerance, dyslipidaemia, hypertension, hyperinsulinaemia, high triglyceride levels, low HDL levels, decreased fibrinolysis and the presence of smaller, denser particles of LDL.[342,343,] In this syndrome, the normal actions of insulin are impaired. Alterations in body fat distribution particularly increased visceral fat may

contribute to these abnormalities.[344,345] A genetic basis has been hypothesized for these metabolic abnormalities[134,346] and chronic sub clinical inflammation has been reported as being part of this syndrome.[135] The tendency to insulin resistance observed in British South Asian adults is apparent in children, in whom it may reflect an increased sensitivity to adiposity.[347]

Studies have reported a role for insulin in the pathogenesis of CAD;[348] tissue resistance to the action of insulin has been hypothesized as a primary factor in the excess rates of both CAD and diabetes in British Asians.[314] Elevations in plasma insulin are known to be an independent predictor of CAD.[349,350] South Asians, in particular, have been reported to have higher levels of postprandiol insulin compared with the Europeans.[351] In the Southall study, major Q waves were strongly associated with glucose intolerance and hyperinsulinaemia in younger South Asians, whereas smoking rates and average plasma cholesterol levels were lower in South Asians compared with Europeans. In a logistic model, controlling for smoking and cholesterol, the odds ratio for major Q waves in Asians compared with Europeans was 2.4 (95% C.I. 1.5, 3.8) adjusting for glucose intolerance and hyperglycaemia reduced this ratio to 1.5 (95% C.I. 0.9, 2.5). These results are consistent with the hypothesis that insulin resistance underlies the high coronary risk in South Asians and strengthens the evidence for a fundamental role of this metabolic pattern in the aetiology of CAD.[18]

However, the mechanisms through which insulin resistance increases CAD risk has been a subject of debate. If insulin resistance underlies the excess risk of CAD in South Asians, it is unlikely that its effect is mediated through blood pressure and conventional lipid related risk factors;[309] insight into this comes from the study of South Asian expatriates, in whom these risk factors are not unfavourably distributed in all the groups of South Asian settlers in the UK who share high CHD risk. Average blood pressures are not high in Muslim South Asians compared with the native British populations. Insulin resistance and the development of cardiovascular disease are probably linked through changes in other lipoprotein and their metabolism. Support for this hypothesis comes from the study of Afro-Caribbeans in UK, who, have been shown to have similar prevalence of type 2 diabetes and insulin resistance but lower rates of CAD;[295] this has been attributed to a more favourable lipoprotein profile in Afro-Caribbeans compared with South Asians;[317] this is further reinforced by recent evidence suggesting that insulin resistance increases small dense LDL particles, which additionally contribute to the increased lipid related coronary risk in this population.[316] Insulin resistance has also been associated with inflammation;[135] C-reactive protein, which is a sensitive marker of systemic inflammation, has been shown to be higher in healthy Asian Indians than in European whites[352] and has been accounted for, by greater central obesity and insulin resistance; this may also highlight another possible mechanism for the increased coronary risk in this population.

2.2.4.vii Obesity: in most epidemiological studies, body mass index has been used as an estimate of overall obesity; such indices however, fail to discriminate body fat from skeletal and muscular mass. The anatomical distribution of fat, on the other hand, appears to be a more consistent and robust risk factor for diabetes and CAD than BMI. Abdominal obesity, a pattern of obesity as measured by waist circumference has emerged

as an even more important risk factor for CHD than total body adiposity, and is more closely associated with cardiovascular disease risk factors studied than overall adiposity as measured by BMI.[139,140] At any given level of body mass index, South Asian men have thicker trunk and skin folds and higher mean waist-hip-ratios than Caucasians; the insulin resistance syndrome, prevalent in South Asian populations is associated with a pronounced tendency to central obesity.[309] A recent study comparing generalized and regional obesity between adult White and Pakistani expatiate men showed that mean BMI was the same in both the groups whereas Pakistani migrants had significantly more abdominal obesity relative to total adiposity.[353] Recent studies have identified that the simultaneous measurement and interpretation of simple variables, such as waist circumference and fasting plasma triglyceride concentrations, could be used as an inexpensive screening tool for the identification of men characterized by the athrogenic metabolic triad;[354] this may be particularly relevant in the South Asian context. Control of central obesity and greater physical activity offer the best chances for prevention of diabetes and CAD in the South Asian population.

2.2.4.viii Psychosocial factors: investigation of ethnic differences in psychological factors associated with CAD have revealed that South Asians suffer from higher levels of depression, higher negative supports, less social support at work, more effort reward imbalance and higher hostility levels compared with Whites.[355] Expatriate South Asians have additional stresses in the host country related to social class, cultural isolation and racism in addition to the known stress related risk factors for CAD operating in the host country. Native South Asians, however, may have a whole host of, as yet poorly identified and measured psychosocial and economic stress factors.[356,357] Changes in the South Asian society such as urbanization, break down of family and social support systems, shift from a rural agrarian to a service and manufacturing economy while facing acute demographic and economic pressures are expected to increase both universal and culturally specific CVD stresses.[358]

2.2.4.ix Physical characteristics: the association between size at birth and CAD was examined by a study in India; low birth weight, short body length at birth, and small head circumference at birth were associated with raised prevalence of disease. The highest prevalence of disease (20%) was in people who weighed 5.5 lb (2.5 kg) or less at birth and whose mothers weighed less than 100 lb (45 kg) in pregnancy.[359] Data from this and other studies carried out on other populations is convincing but in many of the studies, the number of subjects is small. There is also, always a question of selection bias especially relating to perinatal mortality that is often prevalent in malnourished populations; moreover not all studies done on those born during war, famine and siege confirm these findings. The associations between reduced foetal growth and conventional risk factors for CAD do not explain the association between reduced foetal growth and CAD, which indicates that this association must be partly mediated by processes other than known risk factors; this is consistent with the fact that known risk factors cannot explain the high risk of CAD in this population. The hypothesis needs to be further tested in South Asians. Economic and gender disparities seem to predict that the above risks will persist in South Asia with current and future cohorts facing intrauterine nutritional stress and comparative excess of nutritional risk factors later in life.

2.2.4.x Clotting factors: compared with data on White populations, there have been relatively few studies on the thrombotic component of vascular risk in South Asian subjects. Serum fibrinogen which is an independent risk factor for CAD has been found to be higher in South Asians compared with White populations in some,[286,360,361] but not all studies. [362] Data for PAI 1 in South Asians is also unremarkable; PAI 1 was earlier thought to be important since higher PAI 1 activity, by attenuating fibrinolysis, was thought to contribute to the excess macro vascular risk in South Asian subjects with type 2 diabetes. Evidence for this came from the observation that both PIMA Indians living in Arizona and immigrant South Asians living in the UK have a high prevalence of type 2 diabetes and hyperinsulinaemia but PIMA Indians have a low prevalence of and mortality from CAD. Studies however revealed that, although PAI 1 levels are higher in South Asians with type 2 diabetes relative to other ethnic groups; this difference did not account for the differences in the prevalence of CAD amongst both the populations and between diabetics and non-diabetic subjects. [363,364]

Results of a recent study, which was carried out on a clinically healthy Asian population showed a different pattern of thrombotic risk, manifested by increased fibrinogen and PAI 1 activity in Asians irrespective of gender, while levels of VII C and factor XII were found to be lower in this population.[365] The latter findings of the study go against the hypothesis that higher levels are associated with vascular disease. It is possible that factor VII has different effects on vascular risk on different populations; levels of factor VII are also strongly related to genotype; there is increased frequency of the Gln353 allele in Asians, which is associated with 20% lower Factor VII levels.[366] A similar argument may relate to the lower levels of Factor XII in Asian subjects.[367]

2.2.4.xi Homocysteine: most of the data on homocysteine is representative of the developed world population, from which the diet of South Asians is known to differ appreciably. Studies on expatriate South Asians have revealed that plasma concentrations of homocysteine tend to be higher compared with the White[286, 368,369] and East Asian populations.[370] In UK, a parallel case-control study, (one in European and one in Asian Indians) was carried out in which, fasting and post load homocysteine, vitamin B12 and folate concentrations and conventional CVD risk factors were measured. Fasting homocysteine concentrations were 8% (95% C.I. 3-12) higher in cases compared with controls, in both ethnic groups. The odds ratio of CAD for a 5 µmol/l increment in fasting plasma homocysteine was 1.3 (95% C.I. 1.1, 1.6) in Europeans and 1.2 (95% C.I. 1.0, 1.4) in Indian Asians and the association between fasting plasma homocysteine and CAD was independent of conventional risk factors in both ethnic groups. Among the controls, fasting homocysteine concentrations were 6% (95% C.I. 2-10) higher in Asian Indians than in Europeans, a difference that could explain about 20% of the excess liability of this racial group to CAD.[371] This difference could be explained on the basis of the lower vitamin B12 and folate levels in Asians.[372,373] In other studies in expatriate South Asians, there was no evidence that homocysteine played any part in the differential ethnic risk in CHD; however ethnic differences for vitamin B12 and folate were demonstrated; Indians had particularly low levels.[374] Prolonged cooking of vegetables, which is common practice in many Asian Indian households, may destroy up to 90% of the folate content.[375,376] Knowledge of the fact that modification of dietary patterns can have substantial effects

on fasting levels of total serum homocysteine opens new doors to evaluate this hypothesis further in the South Asian population.[377]

2.2.4.xii Inflammation: an infectious basis to atherosclerosis may in part be contributory to the geographical and temporal variations in prevalence of CAD. Most of the work on inflammation and infection, on the other hand, has been carried out in the developed world settings. If general infective load is important, then the relationship of microorganisms to CAD in the subcontinent may be more complicated than that seen in temperate climates in the developed world. A recent study from Sri Lanka does not strongly support the hypothesis that Chlamydia pneumoniae infection may be linked to CAD; results suggest that infection may be linked to CAD through the interaction with some of the known risk factors such as blood lipids, diabetes and smoking.[378] However, CRP, which is an independent risk factor for CHD has been shown to be higher in healthy Asian Indians than in European Whites and has been accounted for by greater central obesity and insulin resistance;[352] adiposity is also known to be a major determinant of CRP,[379] highlighting a possible mechanism for the increased coronary risk in this population.

2.2.4.xiii Migration: the excess mortality and prevalence of CAD in South Asian immigrants may be attributable to the effect of migration itself. Some of these factors associated with migration have been highlighted by comparing a randomly selected group of migrants from the Indian Subcontinent in UK and their siblings living in India. In this study, body weight, serum cholesterol, and blood pressure were found to be high in Indians who migrated to UK. It was hypothesized, that with migration there is a trend towards the acquisition of westernised behaviours, therefore the increase in serum cholesterol after migration unmasks the underlying genetic risk of CAD conferred by high serum Lp(a). The study also showed that native South Asian populations have decreased insulin sensitivity. Whatever the explanation of insulin insensitivity in Indians, the likelihood of increase in insulin resistance, its expression as frank diabetes and glucose intolerance probably increases on migration to more affluent countries.[321]

2.2.4.xiv Ethnicity: it is not known whether ethnicity itself is a risk factor or whether coronary risk in people of South Asian origin operates through conventional risk factors. The existing evidence in favour of an aetiology linked directly or indirectly with the metabolic syndrome necessitates the identification of its genetic origin in South Asians. The effects of the metabolic syndrome and its progression to either type 2 diabetes, or causation of CHD, which is influenced by increasing age, weight gain and physical inactivity, also highlight the role of environmental factors.

2.2.5 Issues

There are several questions that emerge in the overall context of the increased coronary risk of South Asian immigrants. Firstly, it was previously felt that estimates of South Asians excess risk are imprecise,[380] as only prevalence, mortality and health care utilization data were presented. However, the recently available incidence data from

Singapore, comparing Asians with Malays and Chinese has confirmed the increased susceptibility of Asian Indian males to CAD.[287] Secondly, data on prevalence is further confounded by problems such as reluctance or delays in access to care;[381] however it seems unreasonable to think that the reported increase in CAD risk may be entirely due to issues related to access to care. [292]

It has also been pointed out that Asian communities in Britain differ in religious, cultural, geographic and genetic backgrounds and can be seen as heterogeneous groups; it has also been demonstrated that different groups are not similar with respect to conventional coronary risk;[307] however, despite this, different groups are known to share mortality from CAD that is 50% higher than that of the national average.[310] In UK, sir names on death certificates and districts of residence have been used to distinguish communities. [382]

And lastly, the belief that except for insulin resistance, South Asians have lower levels of risk factors compared with Europeans is incorrect. Conventional risk factors are highly prevalent in South Asians, but fail to fully explain the increased risk in this population.

2.2.6 Conclusions

South Asian ethnicity is associated with a high current and projected risk of cardiovascular disease in expatriate and native populations. This may be partly explained by the fact that South Asians have high prevalence of some conventional and novel risk factors. In this context, knowledge of the fact that for any given rate of atherosclerosis South Asians have higher prevalence of CAD has important implications and necessitates the identification of risk factors for atherosclerosis and risk factors for acute coronary events (thrombosis) as two separate entities. Ethnicity or some unmeasured or as yet, unknown proximate and remote risk factors linked to ethnicity, not included in the typical classical high risk composite seen in the Caucasian model might mediate the effect of atherosclerosis on the risk of clinical cardiovascular disease. Many of these risk factors may have additive or synergistic effects, as in the Caucasian high-risk profile; many, however, may be acting fairly independently in promoting atherosclerosis, thrombosis, and resulting CVD. These assumptions are based on detailed biochemical and anthropometric studies done primarily on South Asians living in industrial societies. However, the changing environmental processes of urbanization, migration and acculturation acting upon, a susceptible genotype may influence them. The susceptible genotype too, has not been well defined and may not necessarily be the same in all south Asian population groups. There is therefore a need to re-think the approach towards identifying and mediating CVD risk in the South Asian population.

2.3 Epidemiology of Coronary Artery Disease in Pakistan

It was back in the early nineteen sixties, when Pakistan existed on the map of the world as two geographic entities, West and East Pakistan, that studies from West Pakistan reported significant increase in the number of patients hospitalised for the manifestations of CAD.[383,384] Over the last half a century, modest efforts at collecting risk factor data

have highlighted a significantly high prevalence of traditional risk factors for CAD in both the urban and the rural areas of Pakistan.

2.3.1 Prevalence of coronary artery disease

There is scarce data on the prevalence of CAD in Pakistan and no data on CAD incidence. In the early eighties, prevalence of ischemic heart disease as evidenced by history was reported in a cross sectional survey, which was carried out in an affluent urban and poor rural setting in Karachi.[i] Prevalence was reported at 1.9% in the affluent and 0.6% in the poor areas; the difference between the two was found to be significant (p-value=0.003).[289] However, this study is likely to have significantly underreported prevalence; reliance on doorstep interviews and ascertainment based on household testimony cannot reflect the true occurrence of disease.

The recently reported Pakistan Survey of Health and Living Conditions in the Elderly,[385] was a cross sectional survey of the health status of individuals over the age of 65 carried out in five towns of Pakistan. In this survey, 7% of the elderly reported having been admitted to a hospital with a heart attack (8% males and 7% females).

More than 50% of patients presenting with an acute coronary event in Pakistan suffer from premature CAD; additionally an unusually high prevalence of acute myocardial infarctions and a higher prevalence of left anterior descending artery disease also points to a more aggressive pattern of disease in this population.[305,386]

2.3.2 Prevalence of risk factors

2.3.2.i Smoking: the National Health Survey of Pakistan 1990-94,[14] categorized tobacco use as being common in Pakistan, with 54% men and 20% women using tobacco in one form or the other. According to this survey, tobacco use increased with age; the highest prevalence rates of smoking the *huqqa* or chewing tobacco were found in men and women aged 65 years and above. The risk of smoking doubled for men between the age ranges of 15-24 and 25-44 years; men in the age range 25-44 years had the highest prevalence rates of smoking. For women, prevalence of cigarette and *beedies* smoking was found to be the highest in the age range of 45-64 years. According to this survey, women were much less likely to use tobacco as was evident by 29% of men smoking tobacco compared with 3.4% women. However use of the *huqqa* and chewable tobacco or snuff was more common among women. Men in the rural areas were more likely to smoke than men in the urban areas at every level of education and economic status; overall 24% of illiterate young rural men smoked compared with 19% illiterate urban men. Similarly, men of low socioeconomic status with less education were more likely to smoke than men of higher socioeconomic status and with higher levels of education.

[i] The coastal metropolitan city of Pakistan with a population of over 10 million.

Smokeless chewable tobacco poses a significant issue in Pakistan. Tobacco is chewed in different forms; this includes the use of *naswar* (orally used snuff) and *pan*. Tobacco is also chewed along with betel nuts and is the major cause of cancer of the oral cavity in the Indo-Pak subcontinent. The prevalence of smokeless chewable tobacco is very high in Pakistan with over 10% of the population in Pakistan using chewable tobacco. Women may underreport the use of tobacco because of cultural prohibitions.

2.3.2.ii Hypertension: according to National Health Survey of Pakistan, 1990-1994 there were an estimated 5.5 million men and 5.3 million women with hypertension in Pakistan. The prevalence of hypertension over the age of 15 years was reported at 17.9%; prevalence increased with age and urbanization with people in urban areas having higher prevalence across all age groups. Over the age of 45 years, the overall prevalence of high blood pressure was one in three. Prevalence among Pakistani females was lower than in males and then crossed over, exceeding that of males after the age group of 35-44 years; such a cross over is also evident in the US but occurs at a later age.

According to this survey, 3% Pakistanis with hypertension had controlled blood pressure, defined as a systolic blood pressure of $\leq$140 mm Hg and diastolic pressure of $\leq$90 mm Hg. Most hypertensives were found to have moderate to mild degrees of hypertension but severe hypertension, defined as a diastolic blood pressure of over 105 mmHg was not uncommon and was identified in 4% of urban women in the higher socioeconomic category. Generally higher socio-economic status is known to be associated with hypertension in the developing countries; in Pakistan this relationship was observed for urban men and rural females. In developed countries this association is reversed with hypertension being more common among the less affluent classes.

In another cross sectional survey of risk factors undertaken in four cities of Pakistan; 2256 apparently healthy individuals between the ages of 15 and 60 years were screened for blood pressure, cholesterol levels and smoking habits; 31.5% of the population were reported to have blood pressure levels above 140/90 mm Hg whereas 11.9% of the individuals had blood pressure levels above 160/90 mm Hg.[387]

2.3.2.iii Diabetes: the National Diabetic Survey, 1994[388] reported prevalence of diabetes and impaired glucose tolerance (IGT) in a rural town in the southern province of Pakistan. Oral glucose tolerance tests were performed in a stratified random sample of 967 adults, of which, 387 were men and 580 were women; aged 25 years and above. The diagnoses of diabetes and IGT were made on the basis of WHO criteria. Prevalence of diabetes was reported at 16.2% in men, of which 9% were known diabetics and 7.2% were newly diagnosed. Prevalence in women was reported at 11.7%, of which 6.3% were known diabetics and 5.3%, newly diagnosed. Prevalence rose with age to a peak of 30% and 21% in the 65-74 year age group amongst men and women respectively. Impaired glucose tolerance was detected in 8.2% men and 14.3% women. Thus, total glucose intolerance (diabetes and IGT combined) was present in 25% of the subjects examined. In this study, central obesity, hypertension and a positive family history were strongly associated with diabetes. The association of central obesity was greater in women than in men. These results were among the first in Pakistan to document that glucose intolerance

in South Asia could no longer be regarded as a problem confined to the migrant communities.

In another cross sectional survey of consecutive households carried out in a relatively prosperous and a poor area of Karachi,[289] information was obtained in 4232 adults; prevalence of known diabetes, as evidenced by history, was reported at 4.5% in the affluent population, significantly higher than 1.8% in the poor area (p-value=<0.001). A maximal prevalence of 25% was seen in the affluent community aged 55-64. Diabetes was more common in females in both populations. The overall prevalence of hypertension was similar in the two areas although significantly more frequent in the middle aged and affluent.

The National Health Survey of Pakistan 1990-94, used random blood sugar of more than 140 mg/dl as one of the criteria for the diagnosis of diabetes. According to this survey, diabetes was reported to be an under recognized and an under recorded cause of death in Pakistan with only 36.3% of diabetics aware of their condition. According to the 1994 estimates, there were approximately 2.7 million diabetics in Pakistan, with only 0.8 million diagnosed. In this survey, diabetes tended to increase with age; prevalence doubled, comparing young rural and older rural women, increasing from 5% in the 25-44 years age group to 12% in the 65 years and over age group. The highest prevalence was found to be among urban females aged 46-64 years. More than one out of six (15%) urban men over 65 years of age was found to be diabetic. Higher prevalence in women was observed in both the urban and rural areas in Pakistan. Urban dwellers were more like to be diabetic than those in the rural areas and this was true for both men and women. Urban females aged 45-64 years were found to have the highest prevalence of diabetes at 18%. Rural females, of the same age, had a prevalence of 7% whereas males aged 45-64 years had a prevalence of 9% and 5% in urban and rural areas respectively. According to this survey, very few Pakistanis had controlled diabetes (less than 3% of those 25 years of age and over). Control was defined as having a blood sugar of less than 140 mg/dl among those taking medication for diabetes. The association between body weight and diabetes was also very clearly demonstrated in Pakistan, with prevalence rates increasing to 25% among urban obese women over 45 years of age.

2.3.2.iv Cholesterol: in the Four Cities study of Pakistan, mean cholesterol value in adults was 180±30.55 mg/dl whereas 31% of adults had total cholesterol levels above 200 mg/dl. The National Health Survey of Pakistan, 1990-94 also classified high cholesterol as being above 200 mg/dl. Cholesterol levels were reported to increase with age; one out of every three urban women over 65 years of age was reported to have elevated cholesterol levels, four times higher than the levels found in urban females aged 15-24 years. The pattern of high cholesterol levels in men was found to be somewhat different; urban males aged 35-54 years had the highest prevalence levels, after which prevalence rate declined. Women were reported to have higher levels of cholesterol than men for all ages and in both the urban and rural areas.

In another study, cholesterol and triglyceride levels were determined in blood drawn after an overnight fast from 388 school children aged 5-19 years from private schools in

Karachi. The mean cholesterol levels ranged from 4.4 to 4.6 mmol/l (170.1 to 177.9 mg/dl) for boys and 4.4 to 4.8 mmol/l (170.1 to 185.6 mg/dl) for girls. The range of triglyceride levels was 1.0 to 1.2 mmol/l (88.6 to 106.3 mg/dl) and 0.9 to 1.1 mmol/l (79.7 to 97.4 mg/dl) for boys and girls respectively. 62% of the girls and 54% of the boys had cholesterol values greater than, or equal to 4.4 mmol/l (170 mg/dl), a level, at which, dietary intervention is recommended for children.[389]

In summary, there is scare data available on CAD prevalence and none on CAD incidence in the indigenous Pakistani population; prevalence of traditional risk factors, however, is known to be very high. In addition, causal and temporal associations between risk factors and CAD have not been established for this population and the role of other and novel risk factors has also not been well defined. It is therefore imperative to define the risk factor profile of this population against the backdrop of the urgent need to prioritise preventive strategies. Ideally, efforts at uncovering the risk factor profile of the Pakistani population should have been undertaken in a well-designed multicentre prospective cohort design; however, issues of cost, time, institutional capacity and time lag made this impractical. Given the constraints and the need to define causal relationships, a case-control study was therefore undertaken to investigate causal hypothesis. The next chapter deals with the methodology adopted for this approach.

3 SUBJECTS AND METHODS

Subjects and Methods

3.1 STUDY OBJECTIVES

To describe the lifestyle habits, anthropometric and biochemical characteristics, as yet undescribed, in patients with coronary artery disease in Pakistan.

To determine the age and sex adjusted differences between individuals with and without coronary artery disease, classified by coronary angiography in the following:

- Demographics
- Conventional risk factors for CAD inclusive of tobacco use, lipoproteins, hypertension, anthropometric and glucose abnormalities.
- Other biochemical risk factors such as apolipoproteins, markers of inflammation, insulin resistance, homocysteine, fibrinolytic parameters, as yet undescribed for this population.
- Other psychosocial and behavioural risk factors such as socioeconomic status and psychosocial stress, as yet undescribed for this population.

To describe perceptions relating to heart health and its risk states, as yet undescribed, in patients with CAD in Pakistan.

3.2 STUDY DESIGN

A hospital based case-control study.

3.3 STUDY SITE

This study was carried out in two hospitals, located at a distance of 10 kilometres serving the twin cities of Rawalpindi-Islamabad in Pakistan. In Islamabad, which is the Federal capital territory, the study was carried out at the Department of Cardiology, Pakistan Institute of Medical Sciences (PIMS), which is an 800 bedded tertiary care hospital serving the urban and the adjoining rural population of Islamabad.

In addition, cases and controls were also drawn from the Armed Forces Institute of Cardiology (AFIC), in the city of Rawalpindi, adjoining the Federal capital. There were no differences amongst the study participants recruited from both these hospitals.

3.4 STUDY POPULATION

Patients, in whom a diagnostic coronary angiography had been performed in the last one month, were eligible to participate in this study. At PIMS, this included all individuals presenting for angiography from December 1998 through to September 2000; all members of

the study population were included in the study. However, since the turnover of patients for invasive diagnostic procedures was low in PIMS, cases were also recruited from AFIC for a period of four months beginning January 1999 through to April 1999; again all members of the study population, within this period, were included in the study. Both the hospitals served the same population.

Within the circumscribed period, in both the recruitment centres, individuals with abnormal coronary angiograms meeting specified diagnostic criteria were sought serially from the study population and were enrolled as cases, whereas those with normal coronary angiograms in the study population were age and sex matched against the cases as controls. Additionally, controls were also sought from AFIC through out the study period.

3.5 RECRUITMENT

3.5.1 Cases

Daily visits were made to the pre-cath ward of the Cardiology Department at PIMS, within the period of the study; patients scheduled for their first coronary angiography were listed. A preliminary review of the medical records was undertaken after the verbal consent of the attending physician to ascertain eligibility for inclusion, based on laboratory parameters, medical conditions and drug history (see exclusion criteria). A preliminary interview, which lasted on an average 5 minutes, was subsequently conducted with the patient, usually in the presence of the principal attendant to take the patients initial verbal consent. Prior to this, a verbal declaration statement regarding the study was narrated; this included explanation of the potential hazards of being a study participant, in particular, the risks of venepunture. In the event of a verbal consent being given, it was doubly checked to fully ascertain that the patient was not on lipid lowering medication; the individual was then enrolled. After the coronary angiography, eligibility for enrolment based on angiographic diagnostic criteria both for the cases and the controls was ascertained and the interview scheduled pre-discharge, the following, morning in a fasting state. Two experienced cardiologists, both with the same level of training and using identical protocols, performed coronary angiograms.

3.5.2 Controls

From within the study population, in both the facilities, all individuals with normal coronary angiograms were enrolled, during the study period. Additionally, at the AFIC, after April 1999, all normal cases were listed on a monthly basis, those that were age and sex matched with the cases were contacted through telephone, at which time, eligibility was also ascertained. In the event of the individual being eligible, consenting and willing to travel to the hospital for the study, an interview was scheduled and the subject was requested to come to the hospital in a fasting state.

3.6 INCLUSION CRITERIA

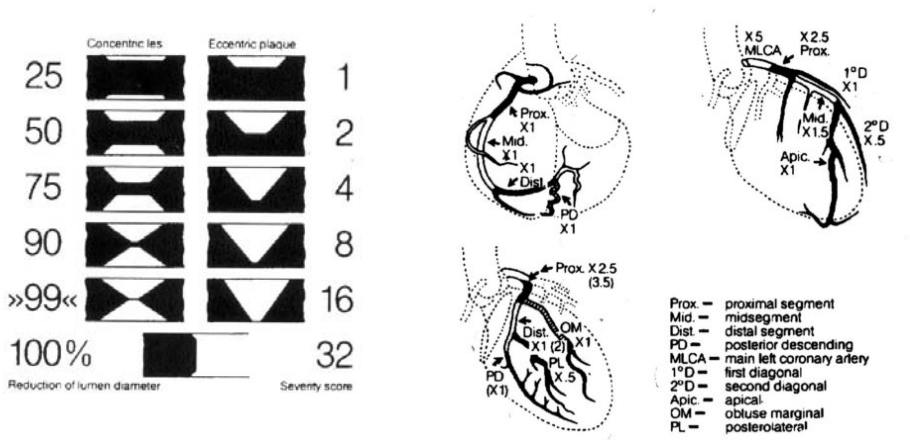
3.6.1 Cases

Patients with abnormal coronary angiograms between the ages of 25-70 years (inclusive) were included in the study.

Diagnostic criteria for an abnormal coronary angiogram: CAD was defined as more than 50% luminal stenosis identified in a minimum of two views, in case of single vessel involvement. Two experienced observers, who were unaware of the diagnosis, scored the angiograms. The degree of stenosis was initially quantitated from the moving cineangiogram by visual evaluation of the percentage of the luminal diameter reduction relative to the calibre of the adjacent normal segment of vessel. In case of a disagreement between the two observers, a third opinion was sought. The degree of stenosis in these vessels was also quantified on the DX Hiline system interfaced with a digital angiography system, both manually and automatically by the densitometric and geometric methods and averaged.

Angiograms were also scored according to the Gensini scale.[390] This method assigns a different severity score, depending on the geometrically increasing severity of lesion, the cumulative effects of multiple obstructions and the significance of their geographic locations.

Fig 3.1 Calculation of Gensini scores on the coronary angiogram



Calculation of Gensini scores involved the initial consideration of the roentgenographic appearance of concentric lesions and eccentric plaques resulting in respectively, 25, 50, 75, 90, and 99% obstruction as well as complete occlusion (100%). The severity of these lesions was then graded on a scale where score 1 was assigned to a 25% stenosis, and the number was doubled as the severity of the obstruction progressed on the indicated scale. The obstruction across each vascular segment was then followed by a multiplying factor such as

X1, X2.5, and so on, depending on the functional significance of the area supplied by that segment as has been demonstrated in Figure 3.1. The author of this thesis who was blinded to other clinical details calculated Gensini scores; efforts were made to ensure quality control and correction for intra-observer variation by validating every fifth Gensini score by another blinded observer.

3.6.2 Controls

Age (within five years) and sex matched individuals with normal coronary angiograms were recruited as controls.

Diagnostic criteria for a normal coronary angiogram: a normal coronary angiogram was defined as one, with no luminal irregularity or stenosis in any of the vessels identified in a minimum of two views reported by two experienced observers unaware of the diagnosis.

3.7 EXCLUSION CRITERIA

3.7.1 Cases

1. Failure to give informed consent
2. Age below 25 years of age
3. Thyroid disease with biochemical evidence of hypo or hyperthyroidism
4. Renal disease with serum creatinine above 150 µmol/l
5. Hepatic disease with serum transaminases 2 times above the normal limit or marked by hypoalbuminaemia
6. Radiologically or pathologically documented malignancy
7. Known bleeding diathesis
8. Patients on lipid lowering therapy, irrespective of the duration of the treatment
9. The presence of coronary stenosis causing less than 50% stenosis

3.7.2 Controls

1. Failure to give informed consent
2. Abnormal resting Q waves on the electrocardiogram (ECG)
3. Abnormal ST segment shifts in the absence of a non-ischemic pathology
4. Multiform ectopic beats on the ECG
5. Left bundle branch block
6. Thyroid disease with biochemical evidence of hypo or hyperthyroidism
7. Renal disease with serum creatinine above 150 µmol/l
8. Hepatic disease with serum transaminases 2 times above the normal limit or marked by hypoalbuminaemia
9. Radiologically or pathologically documented malignancy
10. Known bleeding diathesis
11. Patients on lipid lowering therapy, irrespective of the duration of the treatment
12. The presence of minimal coronary stenosis

Cases and controls were screened to ascertain their eligibility for enrolment. Appendix A and B: Cases and controls screening out questionnaires.

3.8 CONSENT

A written informed consent was taken. In a standardized informed statement, patients were given background details about the study and were informed of their level of participation. Details were given about the timeline involved, the questionnaire, anthropometry and blood sampling. The quantity of blood to be drawn was specified and the vacutainer vials shown to the patients, it was emphasized that disposable needles would be used and that a trained phlebotomist would draw blood. Patients were told that all results would be kept confidential and that names would not be entered on the data entry files. Appendix C: Consent form.

3.9 DATA COLLECTION

A structured interview, observations and documentary sources were used for data collection.

3.10 STRUCTURED INTERVIEW

3.10.1 Variables

During the planning stage, variables to be measured were selected and clarified on the basis of their relevance to the objectives of the study. A list of all the characteristics that were known or suspected to affect or cause CAD was made. Before the questionnaire was designed, the list of variables to be measured was constructed, ensuring that variables had clear operational definitions. Subsequent to this, suitable questions were formulated which had face validity as a measure of these variables. To enhance validity, multiple questions were designed on the same topic. In addition to variables with obvious relevance to the study objectives, other variables were also included; these included universal variables, such as age, sex, ethnic groups, and religion and measures of time, i.e. the date the patient entered the study. In addition to these demographic variables, over 200 variables were identified as being relevant for measurement with respect to the objectives of the study. These included various dietary components, measures of stress, social class and attributes that may be used as indicators of social class, such as occupation, education, income and household crowding index; the place of residence and geographic mobility or migration. Several attributes were also used as indicators of the individual's level of physical activity and exposure to tobacco and the individual's perceptions of health. These variables were too complicated to be measured as single entities, and were therefore, broken up into component aspects, regarded as separate variables and measured independently.

The list of variables to be measured was very long and it was possible to prune it, but since no such data existed for the Pakistani population, it was thought that this dataset would also serve the useful purpose of defining characteristics of the diseased vs. the normal population and therefore most of the relevant variables were included.

Conceptual (dictionary definition) and operational definitions (working definition) of variables were formulated and the scale of measurement to be used in data collection specified, for each variable. Examples of variables measured on different scales include:

Categoric scale

Nominal: e.g. ethnic identity, occupation, birthplace, place of residence, etc.
Ordinal: e.g. stress and diet related variables.
Binary: e.g. possession of items of use, smoking exposure, family history, etc.

Metric Scale

Continuous: e.g. weight, height, blood pressure and all the biochemical variables.
Discrete: e.g. age, units of cigarettes, etc.

Composite Scale: e.g. BMI, waist-hip-ratio, insulin resistance, etc.

3.10.2 Interview

A structured interview was used for collecting information concerning most of the variables listed for investigation; questions were asked orally. Self-administered questionnaires were not used, since they require a certain level of skill and education on part of the respondent, and as our study participants were likely to have low literacy level, that would not have been the preferred approach. In the face-to-face interview, the interviewer was able to maintain the respondent's interest, and was able to allay anxiety if it was aroused. This also enabled the interviewer to show visual aids such as measuring spoons, which were used as a measure of the amount of fat used in cooking.

The study questionnaire colleted data on demographic and socioeconomic status; lifestyle, personal and family history of CAD and risk factors in addition to data on medication. The components of the questionnaire were compiled with the use of previously validated questions included in previous studies. The questionnaire was initially tested on ten cases and ten controls and was modified as necessary. The questionnaire was translated into Urdu (the local language in which it was administered) and subsequently back translated to ascertain that the essence of the questions remained unchanged. It was ensured that all questions had face validity, and it was expected that the respondents would know the answer; questions were clear, non-ambiguous and fair. The sequence of questions involved the inclusion of easy to answer questions in the beginning and leaving difficult, embarrassing and sensitive questions and those that requited a greater interpersonal communication until later. Long questions were avoided; most questions were designed to have fixed alternative responses for greater uniformity and simplicity of analysis. Some however, had open-ended responses such as those on perceptions of heath and stress so as not to loose valuable information. These were subsequently coded without losing information.

Measures were taken to attain complete reliability and to reduce variation to reasonable limits. To this end, clearly defined standardized procedures were developed; questions were asked in a standard manner and the wording and the order of the questions were decided well in advance. Particular attention was also paid to reproducibility or the extent to which similar information is supplied when the question is asked more than once, so as not to generate a bias and to minimize variability of responses.

Factors that could influence the response to a question were identified and taken into account, while working out logistic considerations. A single interviewer was used throughout the period of the study, thus minimizing inter-observer variation in responses as a result of the influence of multiple interviewers. The circumstances of the interview were kept constant; cases and controls were interviewed alone in the research room, dedicated to this activity, in a relaxed atmosphere, early in the morning. A conscious effort was made to interview them at a time when they were in a relaxed frame of mind. Of particular relevance was blinding of the interviewer to the diagnosis at the time of the interview; the result of the angiography was made available to the interviewer after the interview; blinding helped in preventing bias caused by the interviewers knowledge about the subject being a case or a control, and therefore the exposure-suspicion bias was eliminated to a large extent.

Questions were asked in a neutral manner without showing a preference for a particular response; it was made sure that the respondents understood it in the same way. The questionnaire was translated into *Urdu* ensuring consistency in phrasing of questions so that the responses would not generate a bias. The interview took 20 minutes.

3.10.3 Questionnaire

A very extensive questionnaire was therefore developed which looked at a magnitude of demographic, socio-economic, therapeutic, lifestyle, clinical and other variables. Details about various components of the questionnaire and their sources are given in Table 3.1.

Table 3.1: RISKCORN questionnaire

Domain	Method	Source
Age	Date of birth. If unavailable, estimation of age with reference to an index event	National Database and Registration Authority, Pakistan[391]
Ethnicity	Extraction, the place or province of origin	Survey of the Health of the Elderly in Pakistan[392]
Socio-economic status	Education, occupation, income and wealth	INTERHEART study[393] RISKCORN methodology
Physical activity	Work activity, leisure time	INTERHEART study Primary Prevention study[394]
Smoking	Frequency and quantity Duration of exposure Past status Environmental tobacco smoke	European Prospective Investigation of Cancer[395] India case-control study[396]
Nutrition	Cooking method Dietary patterns	SHARE[286] West Lambeth Community Care Trust Project[397] India case-control study
Psychosocial	Acute stress, self perceived chronic stress and depression	Primary Prevention study[398] INTERHEART study
Medical and family history	Diabetes, dyslipidaemia, cancer, hypertension, AMI and other vascular diseases	MRFIT[399]
Medications	Post discharge	RISKCORN methodology

3.10.4 Pre-test of the questionnaire

The questionnaire was pre-tested on 10 cases and an equal number of controls; these were not included as study participants. The interviewer recorded responses for which the questionnaire made provision; in addition, the interviewer also made observations of the respondent's reactions, comments, criticism and suggestions concerning the questions, their sequence, skip patterns and layout of the questionnaire form. The questions were discussed with the respondent after they were answered. It was inquired whether the questions seemed clear, in particular, clarifying the "don't know" answers, to make sure that they were indeed, true "don't knows" as opposed to not answering because of not understanding the question. On the basis of this, it was possible not only to identify the difficult, offensive and hard to understand questions but also to identify unsatisfactory questions yielding a "don't know" answer. The pre-test pointed to a need for changes in the questionnaire, these changes were made and a new version was again pre-tested. These changes have been referred to as the individual variables and questions are discussed and defined in the following sections. Appendix D: Questionnaire.

3.10.5 Individual variables and questions

Name, age and sex: name and sex of the individuals was noted. After initial piloting, the section on name where "first" and "surname" had to be documented was changed since family names and sir names are usually not used in this part of the world. Age of the individual was determined by the date of birth. Majority of the study participants from rural backgrounds with no formal education were unable to recall their exact dates of birth and referred to age with reference to an index event, such as a war or flood; in this situation, approximate age in years was noted.

Occupation and migration: the present or the usual occupation for which, the subject was trained (professional or trade) or work actually performed currently, was noted in an open-ended manner. Past occupation over the years, if different from the current occupation, was also noted. In keeping with the trend of economic development, it is usual for individuals to change the nature of their work, moving from rural to urban areas and from lesser developed to more developed towns, in search for a better living and an improved quality of life; the impact of this movement with respect to coronary risk was thought to be important and therefore past employment status was also noted.

In the same context, place of residence over the years was noted starting from where the individual was born, where he lived during the first fifteen years of life and where he resided for the last thirty years. Places of residence were categorized as village, small town and large town according to definitions given by the census office in Pakistan. This within country, rural to urban migration, which is a part of economic development, was thought to be important with respect to lifestyle changes that accompany this behaviour in the context of coronary risk. Open-ended responses were sought initially and were subsequently coded.

Ethnic identity: the question relating to ethnic identity referred to extraction or the place or province of origin. *Punjabi, Pathan, Sindhi, Mohajir, Balochi,* and *Kashmiri* ethnicity was

documented. Pakistan is divided into four provinces in addition to Pakistan administered Kashmir. Islamabad being the Federal capital territory houses people from all over the country, in addition to being the centre of tertiary referral for Pakistan administered Kashmir.

Education: number of years of education was calculated from the highest class achieved in school or college. Since college and university education is not universal in Pakistan and most people study up to the primary or middle grades, it is customary to refer to the level of education in terms of years of schooling such as 6 or 8.

Socio-economic status: residential details inclusive of address, size of the house, plot size, covered area and the number of people and rooms in the house were noted. While totalling the number of persons in a housing unit, children were taken as wholes and while calculating the number of rooms in the house, bathrooms, kitchen and storerooms were excluded. Additionally, monthly family income and ownership of household items such as a car, bicycle, scooter, television, VCR, telephone, mobile phone, refrigerator, etc. were also documented in an attempt to ascertain socio-economic status. The possession of items such as cattle and tractor were important in determining socio-economic status in the rural areas.

Tobacco use and other addictions: the magnitude of problem posed by tobacco use in Pakistan is more than what the smoking of cigarettes indicates, therefore tobacco use was defined as also including smoking of the *hukka* and *beedies* and smokeless chewable tobacco in the form of *naswar* or *pan*. The current use of tobacco products was quantified in terms of the number of units of each smoked (as number of cigarettes, number of times *huqqa* smoked, number of *beedies* etc.) and the period of time over which this practice continued. In case the respondent was not a smoker, the past history of smoking was sought and the time frame within which, the individual quit smoking and the number of years for which he smoked, were noted; lifetime tobacco exposure was also quantified.

For the purpose of this study, passive smoking exposure was defined as a minimum exposure of 5 minutes during which the individual was unable to avoid inhaling someone else's smoke. According to this criterion, people smoking in the presence of the respondent, such as spouse, co-worker, sibling, children and others, were identified; subsequently, the duration of exposure, in terms of hours per week was calculated. In addition to this, a history of oral smokeless tobacco use was also sought.

As the consumption of alcohol is not routine in the country, questions relating to the same were few and aimed only at ascertaining whether the individual consumed alcoholic drinks, and in case he did, with what weekly frequency. History of drug abuse was also sought. Individuals were however, reassured that answers were confidential and that we were not trying to discover if alcohol and drugs were harmful or not.

Physical activity: the level of physical activity undertaken by the subject was assessed by questions that integrated daily physical activity, exercise, sports, worksite activity and personal chores; in questions relating to exercise, the respondent's type, level, frequency and duration of exercise was ascertained. Additional information was also sought to help in classifying individuals into categories with different levels of physical activity, this included number of hours spent on the feet during the day and the number of hours of sleep.

Diet: a Food Frequency Questionnaire (FFQ) carried out assessment of dietary pattern in this study. Though this subjective method is fraught with methodological problems including recall bias and under-reporting of certain types of food, it nevertheless, gives useful insight into the dietary pattern and was found to be useful for the Pakistani population for which, no such data existed. FFQ have also been used with considerable field success in a recently reported very large Pan-European (EPIC) study.[400] Examples of foods within each food category were listed, in order to provide a description of each category. Individuals were asked how frequently they consumed food from each of the groups.

Meat: weekly servings (the size approximating to a deck of cards) of beef, mutton/lamb, chicken, fish and offal.

Grains: weekly servings of whole-wheat flour *pholka/chapatti, tandoori roti, roghni nan*, white bread, brown bread, *paratha*, rice, pasta, cereals and porridge.

Legumes: weekly servings of lentils and peas.

Vegetables and fruits: weekly servings of vegetables as in curries (cooked) and in salads (raw) and weekly servings of fruits.

Milk: daily servings of full cream, semi skimmed and skimmed milk. In Pakistan, fresh milk is widely used; there are selected urban households, where packaged milk is used but the use of packaged skim milk is negligible. It is standard practice to consume whole milk as it is thought that the source of energy in milk is its cream; therefore it is standard practice, particularly in the rural areas, not to remove cream from milk after it comes to a boil. This form of milk is referred to as full cream milk whereas semi skimmed milk is the form of milk from which cream has been removed after boiling; this practice is most prevalent in urban Pakistani households.

Deep-fried foods: weekly servings of *samosas, pikoras*, etc.

Desserts, sweet snacks: weekly servings of *kheer, halva, mithai,* chocolate, candy, etc.

Salt: a note was made of salt added at the table.

Eggs: the number of whole eggs and/or egg whites consumed per week including the number of eggs used in cooking.

Fat: an inquiry into visible fat intake began with the identification of the currently used form of fat (saturated [*ghee*] vs. unsaturated [oils] and the different types within that). It was inquired whether the household had switched from using *ghee* to oil, and if so, how many years previously. Quantification of fat in the Pakistani diet required looking at dietary fat intake in the context of the eating pattern of the population. There are various sources of visible fat in this form of diet; the principal is in the curry base whereas other sources include fat consumed during breakfast.

The amount of oil used for cooking curry, per person, was also calculated; this involved calculating the number of tablespoons of oil used in all the curries cooked in the house and dividing it by the number of people eating in the house. For that purpose, an earlier count was established to include or otherwise, servants and other permanent guests living in the house. Quantification of the amount of fat added to the curry, on the other hand, was made possible by the standard practice in most households, of using a special wooden spoon for adding oil to the curry as it is taken out from the storage tin. Several such spoons were shown to the individual for reference and in the absence of his knowledge (as in the case of the wife not accompanying a male interviewee) this was followed up by interviewing the wife or the cook at home. Although this method was likely to overestimate fat consumption, not accounting for wastages, it was as close as possible, to the ideal method of quantification in this setting, given the circumstances. In addition, additional visible fat consumed at breakfast was quantified in a similar manner. It is customary in the rural areas of Pakistan to add cream to curry or *roti* taken in the mornings at breakfast; it is also customary to use cooked breakfast and in some instances, *paratha* is traditionally eaten. Adding up all these quantities gave a rough estimate of the number of tablespoons of fat consumed by the individual in a day.

Stress: an inquiry into stresses relating to the economic and cultural background of the study participants was made; this included stresses relating to economic circumstances, spouses, in-laws, situations relating to the daughter's marriages and children's in laws; responses to these were sought on an ordinal scale. In addition, the occurrence of a stressful event a year prior to the onset of the illness was also noted; for these, responses were measured on a binary scale.

Medical section: the questionnaire also included a brief medical history to outline the coronary risk status of the individual. It was also inquired whether the patient was hypertensive and/or a diabetic with provision for a "don't know" option. The last three blood pressure recordings and last fasting and random sugar and cholesterol readings were also noted. In addition, the mode of glucose checking was noted as were the treatment details for diabetes. A note was also made in particular, of a past history of stroke and a systemic inquiry was conducted for complaints relating to cardiovascular diseases.

Family history: a note was also made of the diseases that the parents and the siblings suffered from.

Hormone replacement therapy: it was noted whether women were post-menopausal and if so, were they on hormone replacement therapy (HRT).

Perceptions of health: this section relates to perceptions of health with respect to coronary risk. Questions sought answers to causes of hypertension, obesity, blood pressure, and CAD and their interrelationship in an open-ended fashion. A note was made of the respondents' perceptions of the causes of heart disease and remedies for the same.

Birth weight: birth weight of the individual based on distant second hand recall was noted.

3.11 OBSERVATIONS AND MEASUREMENTS

3.11.1 Techniques of observations

Several techniques of observation were also used as methods of data collection; these varied from simple measurements to more sophisticated techniques and included:

1) Measurement of waist and hip circumference with a tape measure
2) Measurement of blood pressure with a sphygmomanometer
3) Measurement of weight by a weighing scale
4) Measurement of height by a height measure
5) Angiography for documentation of CAD
6) Biochemical analysis for biochemical variables
7) Measurement of body fat by the body fat meter

Particular attention was paid to reliability or reproducibility of results; the procedures were therefore standardized. Using the same investigator for all measurements minimized inter-observer variation and performing two or more independent measurements and comparing the findings minimized intra-observer variation; the mean of two or more values was used wherever applicable. High quality instruments were used and it was ensured that they gave consistent measurements. When more than one measurement device was used, they were of the same model and were standardized against each other. Equipment was tested from time to time and quality control measures ensured.

Other than blood pressure, which was taken before and after the questionnaire, in the standing, lying and sitting positions, physical measurements were taken after administration of the questionnaire. Appendix E: Anthropometry record sheet.

3.11.2 Anthropometric measurements

Brachial blood pressure: a standard mercury sphygmomanometer was used. It was made sure that individuals had not smoked for at least 30 minutes prior to the measurement. Blood pressure was recorded from the right upper arm with the subject initially seated, later standing and finally lying supine for at least 5 minutes. The cuff was applied with its lower border 2-3 cm above the anticubital space, ensuring adequate cuff size.

Systolic pressure was determined by the first heard sound (Korotkoff phase I). Diastolic pressure was recorded at the level when the sound just disappeared (Korotkoff phase V). Two measurements were recorded, one immediately before the administration of the questionnaire and one immediately after. The blood pressure cuff was applied before beginning the questionnaire and left on, until the second measurement was taken. The average of the two, to the nearest whole number, was taken as the blood pressure. Both measurements were taken before the subject was asked to change positions for the remaining anthropometric measurements.

Height: standard height was measured with the subject bare foot, back square against the wall and eyes looking straight ahead. When the set square rested gently on the scalp, height was recorded to the nearest 0.5 centimetre.

Weight: weight was measured with the subject barefoot and wearing light clothing, the scale was standardized to "0" before each use and the subjects' weight was recorded to the nearest 500 grams.

Waist circumference: waist circumference was measured to the nearest centimeter using a standard tape measure over the unclothed abdomen at the smallest diameter between the costal margin and the iliac crest. The tape measure was kept horizontal and just tight enough to allow the little finger to be inserted between the tape and the subject's skin. Subject were made to relax with the arms held loosely at sides and were not allowed to consciously adjust waist circumference.

Hip circumference: hip circumference was measured to the nearest 0.1 centimetre, using a non stretchable standard tape measure, measured over light clothing at the level of the greater trochanters (usually the widest diameter around the buttocks). The tape measure was kept horizontal and just tight enough to allow the little finger to be inserted just under the tape.

BMI: body mass index was calculated by dividing weight in kilograms with the square of the height in meters.

Waist-hip-ratio: waist-hip-ratio was calculated by dividing waist circumference by hip circumference.

Body fat analysis: body fat was analysed with the OMRON BF 300 body fat monitor, a device that measures the percentage and total amount of fat contained in the human body. This works according to the bioelectrical impedance analysis method, which analyses the electrical resistance of the body tissues by sending an extremely weak current through the body. Since fat tissues have little to no electrical conductivity it is possible to determine the ratio of fat tissue compared with other tissues. In this way, the body fat meter measures how much of the total weight is made up by total fat and what percentage this is, of the total weight.

3. 12 LABORATORY PROCEDURES

3.12.1 Blood collection kits

The department of Chemical Pathology at St-Thomas' Hospital, London supplied unlabelled blood collection kits; each consisted of:

1) Two lavender top vacuum tubes, (each 10 ml) with EDTA as preservative
2) One blue top vacuum tube, (5 ml) with citrate as preservative
3) One grey top vacuum tube (1 ml) with fluoride as preservative
4) Centrifuge tube
5) Plastic storage vials (2-6 ml)
6) Transfer pipettes

3.12.2 Blood collection, processing and storage

Fasting blood samples were collected from each subject while seated and the tourniquet applied for the minimal amount of time. The anticubital vein was the most commonly chosen site. A total of 26 ml of whole blood was drawn per subject. 5 ml was taken in the citrate tube; 1 ml in the fluoride tube and two 10 ml samples were drawn in the EDTA tubes. Blood from the vacutainer tubes was transferred to the centrifuge tubes, which were allowed to stand for 30 minutes at room temperature; the tubes were subsequently centrifuged at 1500 g (3000 rpm) for 15 minutes until the plasma and the cells were separated.

Plasma from the citrate and fluoride tubes was separated and transferred to storage tubes. All 3 components i.e. RBC, buffy coat and plasma from both the EDTA tubes were separately stored in different transfer vials. The transfer vials were doubly labelled with blood identity numbers. All samples were stored at -40 degrees; an aliquot of EDTA sample was also stored at -70 degrees. On the average, the time between the collection of samples and storage was less than one and a half hours.

3.12.3 Shipment of samples

Samples were shipped in four consignments on dry ice from the project office in Islamabad, to the department of Chemical Pathology at St Thomas' Hospital, London. Consignments were packaged in accordance with the royal mail requirements for shipment of pathological specimens. On all four occasions, it was verified by two independent observers that the samples reached the laboratory in the frozen state.

3.12.4 Laboratory assays

Table 3.2 summarizes details of the laboratory assays, the principles underlying these and the analysers on which these assays were performed. Details of the biochemical principals are appended. Appendix F: Principals of biochemical analysis.

Table 3.2 Laboratory assays and their methods

Assays	Principal	Analyser
Glucose	Enzymatic colorimetric test using the coupled enzyme GOD/PAP method	Cobras Miras S
Creatinine	Colorimetric test using the Jaffe method	Cobras Miras S
Total protein	Colorimetric test using the biurete reaction	Cobras Miras S
Albumin	Colorimetric determination using bromcresol green	Cobras Miras S
Bilirubin	Colorimetric test using the modified Malloy/Evelyn method	Cobras Miras S
CK-NAC	Kinetic UV test	Cobras Miras S
AST	Kinetic UV test	Cobras Miras S
ALT	Kinetic UV test	Cobras Miras S
GGT	Kinetic colorimetric test	Cobras Miras S
Cholesterol	Enzymatic colorimetric test using CHOD/PAP method	Cobas Miras S, Fara II
Assays	Principal	Analyser
Triglycerides	Enzymatic colorimetric test using GPO/PAP method	Cobas Miras S, Fara II

Continued.....

Assays	Principal	Analyser
HDL	Selective inhibition colorimetric assay, Separated by direct anionic detergent method	Cobras Mira S
Lp(a)	Nephlometry	Behring BN2 analyser
Apo A1 and B	Immunochemical reaction	Behring BN2 analyser
CRP	Nephlometry	Behring BN2 analyser
Fibrinogen	Immunochemical reaction	Behring BN2 analyser
PTH	Two-site chemiluminescence	Nicholas advantage chemiluminescence system
Insulin	Two-site chemiluminescent enzyme labelled immunometric assay	Immulite automated analyser (DPC)
Homocysteine	Isotope dilution electro spray tandem mass spectrometry	-

3.12.5 Calculated laboratory parameters

Low density lipoprotein cholesterol: LDL was calculated by the formula:
LDL= TC-HDL-(TG/2.13), when triglycerides were less than 5 (as in all cases).

Insulin resistance: the HOMA-R parameter of homoeostasis model assessment was used for the estimation of insulin resistance.[401] This parameter is quoted as:

Resistance=insulin/22.5Xe to the power (-ln [glucose]), which rearranges to: Resistance=glucose X insulin/22.5. This expression as the product of fasting glucose and insulin yields a tangible concept of insulin resistance syndrome as it applies to patients and assists in the identification of pre-diabetic states.

Small LDL: was calculated by the LDL/apolipoprotein B ratio.

Log of values: log of Lp(a) and CRP was taken as these were found to be non-normally distributed.

3.13 USE OF DOCUMENTARY SOURCES

Hospital records were used as documentary sources for ECG, previous blood pressure readings and blood sugar values.

3.14 DATA MANAGEMENT

Data was initially recorded in the study folder, which consisted of a consent form, a case or control screening out questionnaire, the lifestyle questionnaire and the anthropometry record sheet. The latest ECG was taken from the hospital records, photocopied and included in the file; subsequently results of the coronary angiogram were also included. Gensini scores were calculated on the margins of the hard copy of the angiography report. After the initial compilation of the data, missing data was sought.

To facilitate computer processing and analysis, codes were assigned to categories of variables with nominal, ordinal or dichotomous scales. Standardized codes were used wherever possible. For "yes" and "no" questions, 1 was allocated to all yes questions and 0 to all no answers; 9 was reserved for unknown and 8 for un-applicable. Qualitative responses were coded later with attention to detail so as not to loose valuable information.

Data was subsequently entered in the data entry file using SPSS version 7.5. An extensive list involving more than 200 variables was generated from this study. After initial data entry, data was cleaned. A note was made of isolated values such as outliers and extreme values; these were checked against original records to make sure that these data values were not the result of errors in coding or data entry. Missing data was also handled; variables for which, more than 10% of the data was missing were not analysed and included in the results.

3.15 STATISTICAL CONSIDERATIONS

3.15.1 Sample size

For the calculation of sample size, a conservative estimate of prevalence of exposure to risk factors was assumed for controls, despite the knowledge that prevalence of risk factors for CAD is high in the adult population of Pakistan. This was done in order to avoid a small sample size. Sample size was calculated on the following assumptions.

Risk factor	Prevalence in controls (P_o)
Hypertension (at age 15 and above)	17%
Hypertension (at age 45 and above)	33%
Smoking	23%
Diabetes	5%
Hypercholestrolaemia	12.6%

Prevalence of exposure among controls, which gave maximum sample size from the above list, was used with the following assumptions:

α = 0.05 (two-sided)

β = 0.20 (power=80%)

R = 2.5 (matched odds ratio of 2.5 expected)

Case to control ratio = 1:1

Correlation coefficient of exposure between matched cases and controls=0.20 (suggested by Dupont)

P_o = 10% (prevalence of exposure among controls)

The required sample size obtained using Dupont (1988) formula was 199 cases and 199 controls.

3.15.2 Software

Statistical analysis was conducted using SPSS version 7.5 for windows 98 and GB Stat 7.0 Dynamic Microsystems (Silverspring, Maryland, USA).

3.15.3 Statistical analysis

As part of the statistical analysis variables were initially examined separately, following which, pairs of variables were examined. Data was analysed using both statistical and graphic procedures and was finally tested against hypothesis; details of various procedures applied and the results obtained are discussed.

The first step in statistical analysis involved summarizing data values and examining frequency distributions of all variables. Description of the baseline characteristics of the study participants was a novel exercise, which gave an account of the demographic and risk factor characteristics of the Pakistani population accessing tertiary care for invasive evaluation; such data, was previously non-existent for the Pakistani population. Frequency distributions influenced subsequent steps in the analysis and detailed studies of relationships were not carried out in areas where cases and controls showed no difference. While summarizing data, the observed counts were changed to percentages; percentages were based on valid responses. Graphical displays were made to eyeball data as information in the frequency table is easier to see if turned into a graphical display.

A variety of statistics were used to further summarize information in a frequency table; mean, mode and median were used as measures of central tendency. Frequencies were generated and means were computed for variables measured on the continuous scale and compared between cases and controls. Trim mean and median were particularly important for variables with extreme values. Percentiles and quartiles were also computed from the frequency table. Range, variance, standard deviation and inter-quartile range were used as measures of variability. The aim of the statistical analysis, however, was to test causal hypothesis, therefore the major part of the analysis involved pairs or sets of variables looking at exposure and outcome.

Conditional multiple logistic regression analysis was used for building the final model, adjusting for all the potential risk factors and description of effect modification. The following steps were followed in the selection of variables for the final model.

Univariate analysis using simple conditional logistic regression of each variable was carried out and matched odds ratio with 95% confidence interval was obtained. In case of binary or ordinal factors, a category with minimum risk was taken as reference category. In case of continuous factors, initial quartile analysis of each factor, was carried out; based on this approach, new variables were generated and prefixed with “q” (for quartile), with cut off values at quartiles. Line graphs were next plotted for each variable, to see the trend for each quartile point. Trends apparent from the line graph influenced the decision to use a variable

as either continuous or as categoric; in case of a clear cut increasing or a decreasing trend, the variable was used as continuous. However, in the absence of a clear cut increasing or a decreasing trend, unconditional logistic regression was used by taking one quartile point as reference and by assessing the significance of differences in odds ratios. If there were no differences in two adjacent categories, data from them was merged provided the new categories were explainable and logical; a new variable was generated in this case and was prefixed with "r" (for recoded). In case where differences in odds ratios were observed, the significance of differences was taken into account, if the differences were insignificant, the variable was still used as continuous, whereas in the case of the differences being significant, quartile analysis of the variable was carried out.

For all continuous variables, distribution of the data was noted and in the case of non-normally distributed data, log of the variable was used. In case of deviation from the line of normal distribution, two options were available. In the first option, quartile analysis was carried out if data was found to be meaningful whereas the other option involved comparing the continuous variable with the log transformation of the variable; in the absence of a significant difference, data was used as continuous, whereas in the presence of significant differences between the two, the variable was used in the log form or it was still used in the quartile form if it was thought to be meaningful. Natural log of Lp(a) and CRP was used as they were found to have a skewed distribution.

The next step involved selection of the variables for inclusion in the final model. Any variable whose p-value was found to be less than 0.2 on univariate analysis, or was otherwise, biologically, thought to be meaningful was included in the model. Any factor for which, the p-value was greater that 0.2 and was not biologically meaningful, was excluded. Amongst factors with a p-value less than 0.2, those with sparse data (e.g. stress variables) were also excluded. Factors were also excluded from the model if they distorted the model due to very high standard error for the coefficient. For model building, the best subset selection technique was used, starting with the two most significant variables and adding other variables accordingly. A number of models containing all possible combinations of variables that were significant, according to defined criteria were tried; models were compared through the likelihood ratio test. Multicollinearity, confounding and possible interactions were also assessed at this step.

In addition to the differences in exposure between cases with disease and controls without disease, the relationship between different proximate and remote risk factors was also analysed; in this case, these were considered as dependent and independent variables respectively. Linear regression and correlation was next used by using baseline data or log transformed data, depending on whether individual analytes showed a Gaussian distribution, to determine the relationship of Gensini scores and the exposure variables measured on the continuous scale and the nature of the relationship; prior to this exercise, the existence of a non-linear relationship was excluded by eyeballing scatter plots. The statistical significance of the relationship was computed by calculating p-values and regression coefficients.

An independent line of analysis was also carried out to compare risk factors between males and females in the study; prior to this exercise, box plots and histograms were generated to eyeball data. For variables measured on the continuous scale, in the case of normally

distributed data, independent samples t test was used for comparing mean values between males and females, while in the case of skewed data, the non-parametric 2 independent samples procedure (Mann Whitney U test) was applied for the same purpose.

Differences were also sought, within study participants, belonging to different ethnic identities and between individuals with different vascular anatomies; this involved comparison of means between more than one groups. For continuous variables, box plots were initially generated to separate normally and non-normally distributed data. One way analysis of variance (One-Way ANOVA) was used to determine the statistical differences in mean values where data was normally distributed whereas the non-parametric K independent samples procedure (Kruskall Wallace test) was used in instances where the data was non-normally distributed. For variables measured on the ordinal and nominal scales, involving more than one response, chi-square was applied for significance testing to ascertain differences; in the event of the number of subjects being less than 5, even in the case of a single cell, fisher's exact test was applied for significance testing. In case of variables measured on the ordinal scale, such as in the case of number of servings per week of food items in the FFQ and levels of stress, chi-square for trend was looked at to ascertain the differences in shifts of pattern.

3.16 ETHICAL CONSIDERATIONS

The hospital ethics committee of the Pakistan Institute of Medical Sciences approved the study in August 1998. The study was not seen as posing a hazard to the well being of patients; it was also considered ethical to perform this study at the expense of other research activities of the department, warranting the requited expenditure of time, manpower and money.

3.17 PROBLEMS WITH CASE CONTROL STUDIES

The major part of the work in the thesis involved case-controlling. The basis of case-control studies, which by definition are retrospective and cross-sectional, is the comparison of one group with another, regarding one or more characteristics of interest. Cases or subjects with the disease of interest are matched to a greater or lesser degree and the groups compared for characteristics of interest. Whereas case-control studies provide a valuable tool in the understanding of aetiological factors in disease, there are advantages and disadvantages of this methodology.

A major advantage is that case-control studies can be done rapidly and inexpensively in comparison to longitudinal cohort investigations. Secondly, case-control studies are suited to the relative advantage of the case-control approach. Thirdly, case-control studies allow the evaluation of several different aetiological factors both as independent and interacting causes.

There are also several disadvantages of case-control studies, which may not be suitable for the study of rare exposure to an aetiological agent; case-control studies are, however, suitable if exposure causes a high proportion of a particular disease, such as in this case. Secondly, case-control studies allow estimation of relative risk, but not absolute rates and thirdly, such

studies are susceptible to biases. Particular attention was paid to mitigate biases in this study; these have been discussed below.

The source of the control group is also a major factor in case-control studies; the choice of controls in this study has been discussed below in detail.

3.18 DISCUSSION ON THE METHODOLOGY

Ideally efforts at uncovering the risk factor profile of the Pakistani population should have been undertaken in a well-designed, multi-centre prospective cohort design; however, issues of cost, time, institutional capacity and time lag made this impractical, particularly as there is an urgent need to formulate preventive strategies in the face of the current and foreseen cardiovascular disease burden. Given the constraints and the need to define causal relationships, a case-control study was therefore undertaken to investigate causal hypothesis.

3.18.1 Diagnostic criteria for coronary artery disease

For decades, issues in studies establishing a risk factor-causal association with CAD have centred on the diagnosis of CAD. Diagnostic criteria used for CAD in studies by the World Health Organization[399] and the United States National Institute of Health,[402] have been historic evidence (documentation) of myocardial infarction, angina and previously diagnosed disease, affirmative response to the Rose questionnaire and electrocardiography findings, some identified with changes on the Minnesota code. There are however, several problems with this approach. Historical evidence in CAD is known to have a very low sensitivity and specificity; Minnesota coding of ECG is fraught with methodological flaws; the validity of Rose questionnaire in the diagnosis of CAD in population surveys has been questioned,[403] and stress evaluation has unacceptable levels of false positives and negatives for it to be used as a diagnostic criteria. Invasive angiographic evaluation on the other hand, is the gold standard for the diagnosis of CAD; whereas issues of cost, ethics and logistics preclude its utilization as diagnostic criteria in population studies, it was possible to use it in the setting of a hospital based, case-control study design.

To recruit prospective cases of CAD, documented by coronary angiography, in a case-control design, a hospital based setting needed to be chosen with those within the study population having normal coronary angiograms recruited as controls after matching for age and sex. Unlike studies,[404] that have classified prevalence of CAD into definite, possible and unreported according to preformed criteria, in this study, the key advantage lies in using the gold standard for the diagnosis of CAD and thus, problems of misdiagnosis associated with the use of less specific diagnostic modalities such as, for example, ECG have been avoided. The study design involving a hospital based sample of those, who had already had an invasive test, which formed the basis of their inclusion in the study, obviated ethical issues around the inclusion of invasive diagnostic criteria in population based studies. It is hoped that in future, more accurate non-invasive tests with a similar sensitivity and specificity as coronary angiography, e.g. ultra fast CT will be available that will eliminate ethical issues in the

diagnosis of CAD through invasive methods in population based studies, and which will make accurate diagnosis feasible in a population based setting.

3.18.2 Prevalent versus incident cases

The inclusion of incident rather than prevalent cases is traditionally important for a number of reasons, some but not all of these considerations apply here. In incident cases, the time lapse since exposure to the suspected causal factors is classically thought of as being shorter. This does not apply to the CAD-risk factor exposure paradigm, as this is essentially of behavioural and genetic origin, and does not lend itself to time distinctions. The same holds true for the time relationship between onset of disease and exposure to these factors, which is traditionally thought of as being clearer in incident cases. The use of incident cases does however avoid the prevalence incidence bias to some, though not to the fullest extent. If prevalent cases of CAD based on an established diagnosis of prior major acute event were enrolled as cases in this study, then those that recovered without any residual ECG criteria would have been underrepresented and those that died suddenly not at all.

3.18.3 Matching

To avoid differences in characteristics of the case and control group, which could obscure or distort the associations being studied, the control group was matched with the case group, ensuring similarly with respect to possible confounding factors. Matching reduces the confounding effect and under certain conditions, adds to the precision, with which, the association under study is estimated.

In general, a case-control study should have only one control group and a second should be added only if the first has a deficiency, which can be offset by the second group. In this case, verification of the disease free status of the control group, by using the gold standard for diagnosis obviated the need for another control group. Biases related to the definition of the control group were also obviated as controls had, as in the ideal theoretical situation, undergone the same investigative procedures as the cases; conventionally in case-control studies, this is seldom achievable, but was attained due to the specific design of this case-control study.

An equal number of age and sex matched controls were recruited in this study. Since cases and controls were matched for age, it was not be possible to study the relationship of disease to age and thus a confounding variable that was controlled by matching, could no longer be studied as an independent variable. Also, it became difficult to reach useful conclusions with regard to associations with variables that are closely linked with age. However the effect of age as a modifier of other relationships could still be studied. i.e. the effect of smoking with CAD in different age groups.

3.18.4 Elimination of biases

Traditionally, there are numerous sources of bias in a case-control study; efforts were made in this study to mitigate these; with all members of the study population enrolled, a high response rate and very few dropouts and non participants, several biases such as the non-response bias, non-participant bias and drop-out bias were eliminated to a very large extent. An attempt was also made to eliminate several other biases; a single interviewer was used throughout the period of the study, thus minimizing inter-observer variation. There were also efforts made to ensure quality control and corrections for intra-observer variation. In addition, blinding the interviewer to the diagnosis at the time of the interview helped in eliminating the exposure-suspicion bias; this was aided by strict standardization of the anthropometric and laboratory protocols and a single observer.

3.18.5 Statistical considerations

Statistical techniques have been developed in case-control studies which allow both multivariate analysis and stratified analysis for describing associations, for evaluating interactions and evaluating and controlling confounding variables. In a stratified analysis, data is stratified in terms of a variable, which differs between the cases and controls and the groups are compared within strata. Since within strata the confounding variable does not differ within cases and controls, any estimate of risk attributable to a parameter of interest between these is no longer confounded by the initial difference. This technique is exemplified by the use of the Mantel-Hansel statistic. A second approach is the use of multivariate techniques, mainly logistic regression, where adjustment is performed for confounding variables prior to the study of factors of importance; this approach was used for analysis in this study.

Case-control studies generally present results in the form of odds ratio, which represent the excess risk of exposure to an aetiological factor in cases compared with controls, where there is no such exposure. Hypotheses are accepted on the probability (p) value associated with the exposure frequencies. Unfortunately, the p-value may be positive or negative by means of chance alone and no estimate of the role of chance is provided in a study with a negative result; secondly, the p-value reflects both the study's size and observed strength of association, but does neither well. A superior alternative is the computation of confidence intervals around the point estimate of effect; this allows the reader to see the strength of association, as well as inferring the reliability of the result; confidence intervals have been presented in this thesis wherever applicable.

4 RESULTS AND DISCUSSION

Results and Discussion

A. CAUSAL HYPOTHESIS

4.1 RECRUITMENT SITES AND STUDY POPULATION

4.1.1 Results

4.1.1.i Recruitment site 1, Pakistan Institute of Medical Sciences: a total of 405 diagnostic coronary angiograms were performed at the Pakistan Institute of Medical Sciences during the study period (December 1998 to September 2000). Out of these, 115 were enrolled as cases and 59 as controls. Of those that were not enrolled, 34.9% (84/241) were on lipid lowering therapy, 19.5% (47/241) did not consent to participate in the study; 15.4% (37/241) could not be categorized into cases or controls based on criteria specified in the study, 7.5% (18/241) were excluded because of deranged biochemical parameters, in 12% (29/241) participants were enrolled initially but did not complete the study protocol and in 10.7% (26/241), the attending physicians did not give his consent.

4.1.1.ii Recruitment site 2, Armed Forces Institute of Cardiology:

Cases: 123 coronary angiograms were as labelled as being abnormal according to the study criteria, during the period of the study, January 1999 to April 1999; of these, 85 were enrolled as cases. 73.7% (28/38) were excluded as they were on lipid lowering therapy, 23.7% (9/38) did not consent to participate, whereas 2.6% (1/38) were excluded because of deranged biochemical parameters.

Controls: 151 controls were recruited from the Armed Forces Institute of Cardiology during the entire study period, December 1998 to September 2000. Controls were identified from the coronary angiography register; they were contacted by post and through telephone calls.

As is shown in the Table 4.1, of the cases, 57.5% (115/200) were recruited from the Pakistan Institute of Medical Sciences and 42.5% (85/200) from AFIC whereas amongst the controls, 24.5% (49/200) were recruited from PIMS and 75.5% (151/200) from AFIC.

Table 4.1: Recruitment by hospital facility

	Hospital facility	Date recruitment started	Date recruitment ended	Number
Cases	PIMS	December 1998	September 2000	115
	AFIC	January 1999	April 1999	85
Controls	PIMS	December 1998	September 2000	49
	AFIC	December 1998	September 2000	151

4.1.2 Discussion

This study was carried out in two hospitals, one each in the twin cities of Rawalipindi and Islamabad, which are situated in the North Eastern part of Pakistan; Islamabad is the Federal capital of Pakistan with a population of 0.9 million, whereas adjoining Rawalipindi has a population of 0.8 million. The Pakistan Institute of Medical Sciences situated in Islamabad, is an 800-bedded multi-specialty hospital with a turn over of around 250 coronary angiograms per year. At the Armed Forces Institute of Cardiology based in Rawalpindi, on the other hand, more than 4000 coronary angiograms are performed per year. Both the hospitals serve the urban and the adjoining rural areas of the Federal capital territory and together, both these facilities provide invasive diagnostic services not only to the population of the twin cities of Rawalpindi and Islamabad but a large area around it. The cultural, social, economic, demographic and ethnic backgrounds of individuals accessing care in both these facilities were similar.

There were three categories of individuals that accessed Pakistan Institute of Medical Sciences for coronary angiography. Those, that were entitled by virtue of their employment status; this group usually represented all social classes in Pakistan, employed by the Federal Government. Secondly, individuals who were either able to pay or got free treatment through the *Zakat*[ii] scheme. Similarly the Armed Forces Institute of Cardiology also provides services to all categories of individuals; primarily set up to serve all classes within the military, this hospital also serves as a tertiary referral for all classes of individuals and is accessible to the poor and the destitute by virtue of its extended subsidies and *Zakat* schemes. It was an initial concern that the study participants might constitute a selected subset of patients with CAD from a higher socio-economic class since they were able to access tertiary cardiac care; therefore, it was thought that participants might not be typical of the Pakistani or the reference population. However, subsequently, impressions derived from an assessment of the profile of patients accessing care in these institutes and the subsequent analysis of data revealed, that the study population represented all socio-economic groups. At the time of the study, the coronary angiography cost package was lowest in the facilities participating in the study and was therefore likely to be accessed by individuals looking for a low budget option. In addition, results revealed that 38% of the patients were *Zakat* funded[405] and therefore belonged to the lower socio-economic stratum. However, on the other hand, analysis of income of the study participants as a proxy of the socio-economic status gave further insight into the issue and revealed that the average income of the study participants was 30% higher than the average income in Pakistan.

Based on all these considerations, it may be appropriate to infer that the study participants did not exclusively belong to the higher socio-economic class but were a heterogeneous mix of all socio-economic groups with a greater than average income compared with the average Pakistani population. They were therefore, not entirely representative of the reference population. The study population was not chosen because it was typical of a broader

[ii] Islamic charitable relief system, managed centrally by the Government in Pakistan.

reference population to which we wished to generalize the findings, but because of other considerations that have been discussed in detail in the previous section.

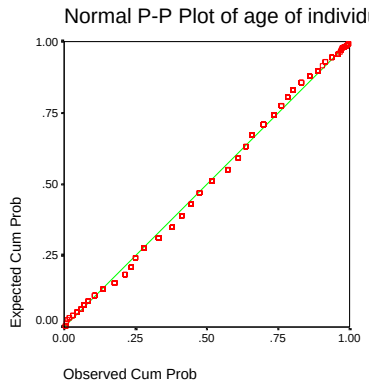
4.2 AGE

4.2.1 Results

4.2.1.i Group ages: mean age of the population was 49.73 (SE=0.48) years, with the median and mode both at 50. Analysis of age by quartiles showed that 25% of the cases were 43 years of age or younger, 50% were 50 years or younger and 75% were 57 years of age or younger.

4.2.1.ii Ages of the cases and controls: cases (mean age 51.27; SE 0.67) were on the average, 3 years older than the controls (mean age 48.22; SE 0.68); the difference was found to be statistically significant (mean difference= -3.06; 95% C.I. 4.93, -1.18; p-value=0.001); however, the distribution of age was found to be normal.

Fig 4.1: P-P plot of age of the study participants



144 people did not know their dates of birth; ages were approximately inferred with reference to important events close to the time of birth recalled by the individuals themselves or their accompanying relatives.

4.2.2 Discussion

Table 4.2 compares mean ages of Pakistani cases and controls in this study with mean ages of native Indian, expatriate South Asian and native Caucasian cases and controls in similar studies. Cases, in this study, were found to be significantly younger in age compared with native Indian and Caucasian cases.

Table 4.2: Mean ages of cases and controls in other angiographic studies carried out on different populations

	RISKCORN population (Pakistan)	Native Indian population	Expatriate South Asian population (UK)	Caucasian population (UK)
Cases	51.27±9.45	56.2±8.8 (p-value= <0.001)	50.7 (p-value=0.40)	55.9 (p-value=<0.001)
Controls	48.22±9.54	47.8±10.5 (p-value=0.50)	50.9 (p-value=<0.001)	51.5 (p-value =<0.001)

Native other Indian populations[406]
Expatriate South Asian and native populations in UK[301]

Because of inherent limitations, age of onset of CAD is difficult to infer even in the setting of well-designed, population-based cohort studies; time of onset of disease is not related to the time of presentation for invasive evaluation, which depends on several factors, including the symptomatic severity of disease, logistics and other factors that influence access to care. Therefore, the age of presentation for angiography as evident by this data, in the overall context of the age of onset of CAD may just be the tip of the iceberg in the general population of Pakistan. Mean age of the population derived from this data points to younger onset disease; this is further reinforced by evidence from studies carried out on expatriate Pakistanis. The observation needs further detailed analysis in this population.

Younger onset CAD has serious implications for a developing country and adds to the health care and lost productivity costs. Large family sizes and the single breadwinner model, make the health of the breadwinner crucial for the well being of the family, in the absence of which, the economic pressure of the household is likely to be transferred to women and children with adverse health economic implications.

4.3 ORIGIN AND RESIDENCE

4.3.1 Results

4.3.1.i Origin: about two-thirds of the controls and three-quarters of the cases were currently residing in urban areas. The difference was not found to be significant (p-value=0.249); Table 4.3 summarizes frequencies.

Table 4.3: Origin and background of study participants

Urban or rural background	Case %	Control %	MOR (95% C.I.)
Rural	27.5	33.0	1
Urban	72.5	67.0	1.28 (0.84, 1.93)
n	200	200	

4.3.1.ii Place of birth: 54.3% of the study participants were born in villages, 14% were born in small towns and 31.8% were born in large towns. Of the cases, 50% (100/200) were born in villages, 14.5% (29/200) were born in small towns and 35.5% (71/200) were born in large

towns whereas of the controls, 58.5% (117/200) were born in villages, 13.5% (27/200) were born in small towns and 28% (56/200) were born in large towns. No significant differences were observed with regard to places of birth between the cases and controls (χ^2=3.175; p-value=0.24). Table 4.4 gives details about the birthplaces of the cases and controls; the odds of being a case were not significantly higher amongst those who were born in towns compared with those born in villages.

Table 4.4: Birthplace of cases and controls

Birth place of the cases and controls	Case %	Control %	MOR (95% C.I.)
Village	50.0	58.5	1
Small town	14.5	13.5	1.28 (0.73, 2.26)
Large town	35.5	28	1.49 (0.95, 2.33)
n	200	200	

4.3.1.iii Migration: the effect of migration from villages to larger towns is shown through a comparison of the place of birth, place of residence for the first 15 years of life and the place of residence during the last thirty years. Amongst those who were born in villages, 83.9% remained in villages till the age of 15 years, whereas 16.2% had migrated to cities. However over the last thirty years, only 24.4% remained in villages while 75.6% had migrated to larger cities.

To determine the differences in the pattern of migration, cases and controls were categorized into those with a predominantly rural background; those that lived in rural areas for the first 15 years of their life and then moved to villages indicating early migration; those that have spent the last 30 years of their life in towns, indicating late migration and those with predominantly an urban background. To develop these categories, 27 possible combinations were made by combining three variables; place of birth, place of residence for the first 15 years of life and place of residence during the last thirty years. All three variables had the same coded response "village", "small town" and "large town" based on the definition of these sites in the Pakistani context. Based on this classification, as is shown in Table 4.5, the odds of being a case were not significantly higher amongst those who were from an urban background, or who had migrated early or late to the urban areas compared with those who lived in the rural areas.

Table 4.5: Background of origin and migration patterns

Migration pattern	Case %	Control %	MOR (95% C.I.)
Rural background	15.1	13.6	1
Early migration	30.2	37.7	0.71 (0.38, 1.31)
Late migration	6.5	9.0	0.70 (0.30, 1.68)
Urban background	48.2	39.7	1.14 (0.63, 2.08)
n	199	199	

4.3.2 Discussion

Overall, more than 70% of those enrolled were urban in origin. This sharply contrasts with the 30:70 urban rural distribution of the Pakistani population, where 70% are based in the rural

areas and only 30% of the population lives in urban areas. This trend amongst the study participants clearly indicates that urban residents had better access to care.

However not all urban residents were born in the urban areas; analysis reveals that 51% of those, currently living in the urban areas were originally born in villages. The effect of migration from villages to larger towns was shown through a comparison of the place of birth, the place of residence for the first 15 years of life and the place of residence during the last 30 years. Amongst those who were born in villages, 83.9% remained in villages till the age of 15, whereas 16.2% had migrated to cities. However, over the last thirty years, only 24.4% remained in the villages while 75.6% migrated to larger cities. This pattern demonstrates that it is after 15 years of residence in the villages that people usually migrate to larger towns; the trend is indicative of migration in search for better opportunities of livelihood and has implications for coronary risk. Whereas an increasing trend of disease was observed with migration to towns, significant differences were not observed amongst those that migrated early and late to towns and those that were predominantly from an urban background, compared with those that belonged to rural areas (p-value=0.19).

Studies carried out on the expatriate population have shown that with migration to more developed countries, there is a trend towards the acquisition of westernised behaviours, consistent with a sedentary lifestyle, high salt and fat and low fibre diet and stresses in the new place of residence. This causes a rise in body weight, blood pressure, serum cholesterol and glucose. The increases in serum cholesterol after migration unmasks the underlying genetic risk of CHD conferred by high Lp(a) and with the increase in body weight, there is deterioration in insulin resistance and B cell function and consequently the manifestation of frank diabetes.[321] Therefore, the acquisition of a risk profile similar to the host community after migration, unmasks the underlying genetic risk of CAD. It is possible that the same hypothesis operates at a much smaller geographic level of migration to the urban areas. The hypothesis needs to be tested further in the Pakistani context.

4.4 OCCUPATION

4.4.1 Results

A breakdown of the current occupational status of the study participants is shown in Table 4.6.

Table 4.6: Job description of the cases and controls

Occupation	Case %	Control %
Professional:		
Doctor	1.5	2.5
Accountant	2.0	3.0
Engineer	4.0	3.0
Paramedic	3.0	3.0
Teacher	2.5	2.5
Lawyer	1.5	0.5
Religious leader	1.0	-

Continued.....

Occupation	Case %	Control %
Managerial/executive:		
Politician	0.5	1.5
Managerial staff	17.0	12.0
Business related:		
Business	14.5	12.0
Service provider:		
Military	4.0	20.0
Police	2.5	1.0
Agriculture related:		
Farmer	4.0	3.0
Production related:		
Manual worker	4.5	1.0
Skilled worker	5.0	7.5
Transport related:		
Driver	2.0	1.0
Looking for job:		
Unemployed	5.5	5.0
Retired/Housewife:		
Retired	10.5	9.0
Housewife	14.5	12.5
n	200	200

Whereas this gave a good overview of the occupational backgrounds of those presenting for angiography, the data was too spread out and unfocused for further analysis, therefore, job categories were developed. According to this, occupations were categorized into: white-collar jobs (inclusive of military personnel, doctors, engineers, teachers, politicians, lawyers, managerial and corporate staff and religious leaders); blue collar and skilled worker category (inclusive of small businessmen, farmers, drivers, accountants, paramedics, police officers, manual workers and skilled workers); retired personnel and housewives were grouped together whereas the unemployed were classed into another category.

Table 4.7: Job categories of the cases and controls

Job categories	Cases %	Controls %
White collar	33.0	45.0
Blue collar	36.5	28.5
Retired/housewife	25.0	21.5
Unemployed	5.5	5.0
n	200	200

4.4.2 Discussion

45% controls vs. 33% cases had white-collar jobs, implying that since controls came from a more privileged class, they had better opportunities to access care. In our sample, more teachers presented for angiography, which contrasts, with the percentage distribution of teachers in the general population; therefore based on this data, it would be inappropriate to say that teachers in Pakistan suffer from a higher rate of CAD as this would indicate a referral bias.

Occupation can influence CAD risk through its interrelationship with several risk factors, foremost amongst these are stress related. Stress factors that directly relate to occupation include effort reward imbalance, lack of social support at work and the type of occupation involving a low control, high demand description whereas other stress factors including depression, anger, anxiety, hostility and hopelessness can also be work related. Studies carried out on White populations in UK have shown that individuals with low control high demand occupations and those suffering from chronic life stresses at work are more prone to developing CAD and are more likely to have adverse risk profiles compared with those who do not. Work description can further interact with coronary risk through its relationship with physical activity. These have been discussed further in the relevant sections.

4.5 SOCIOECONOMIC STATUS

4.5.1 Results

4.5.1.i Monthly income[iii]: controls had a mean monthly income of £209 (SE=20.7) whereas the mean income of the cases was £176.4 (SE=23.17). The difference was not found to be significant (MOR 1.00; 95% C.I. 1,1; p-value=0.234). More cases (35%) compared with controls (23%) however, belonged to the low income categories defined as less than £56 a month, and less belonged to the highest income categories defined as greater than £116 a month (45% vs. 54%); the difference was found to be significant (χ^2=5.92; p-value=0.05).

4.5.1.ii Risk factor prevalence in different income categories: analysis of risk factor prevalence by income levels revealed that individuals in lower income categories had less leisure time physical activity (χ^2=17.38; p-value=0.02) consumed more fat (χ^2=13.63; p-value=0.01) and underwent higher levels of stress (χ^2=12.66; p-value=0.005) compared with individuals in higher income categories. There were however, no differences observed in the status of smoking (χ^2=2.02; p-value=0.35) and the prevalence of diabetes (χ^2=0.002; p-value=0.98). Mean values of variables measured on the continuous scale were also compared between groups with different income levels; there were no significant differences found between systolic (χ^2=0.64; p-value=0.72, Kruskal Wallis) and diastolic blood pressure (χ^2=0.64; p-value=0.72, Kruskal Wallis), total cholesterol (χ^2=2.30; p-value=0.32, Kruskal Wallis) and glucose values (χ^2=0.18; p-value=0.91, Kruskal Wallis). Absence of differences in blood pressure and glucose could be explained, as most of the hypertensive and diabetic study participants were on medication. Diabetics however, belonged to the higher income category; the difference was found to be of marginal significance (p-value=0.07).

There were no associations found between income and systolic (r=0.04; p-value=0.47) and diastolic blood pressure (r=-0.003; p-value=0.96), glucose (r=-0.02; p-value=0.02), total cholesterol (r=0.01; p-value=0.75), triglycerides (r=0.06; p-value=0.24), LDL (r=-0.01; p-value=0.8) and HDL (r=0.04; p-value=0.36). However a significant association was found between log of CRP (r=-0.14; p-value=0.007) with monthly income.

[iii] Conversion rate of Pound Sterling to Pak Rupee as of March 31, 2000, Rupees 86.3 to a Pound

Table 4.8: Mean income levels in study participants with different categories of risk

	Mean monthly income in Pound Sterling (SE)	95% C.I.
Activities while awake:		
Sedentary	218.64	154.80, 282.40
Light activity	186.90	146.10, 215.70
Heavy physical activity	184.60	116.70, 252.41
Fat quantity used per day:		
One tablespoon per day	147.70	115.80, 179.53
Two to three tb. sp a day	250.30	182.90, 317.60
Four to five tb. sp a day	217.30	127.60, 307.14
Six or more tb. sp a day	94.86	75.90, 146.80
Socio-economic stress:		
Never	245.70	184.17, 307.24
Some	172.07	132.76, 211.38
Several period of stress	128.68	92.12, 165.24
Permanent stress	204.55	52.60, 356.51
Ever smoker or not:		
Current smoker	162.31	106.43, 218.19
Past smoker	174.51	130.13, 218.87
Never smoked	217.12	163.04, 217.21
Diabetes:		
No	175.34	149.56, 201.12
Yes	290.71	165.81, 415.60

4.5.1.iii Current residence: 88.8% of the study participants lived in houses, 11.3% lived in flats whereas 38.5% rented their accommodation and 61.5% owned the accommodation. Table 4.9 summarises frequencies; no significant differences were observed between cases and controls. The mean number of people living in the house inclusive of domestic help and children was 8 (8.83 amongst controls and 7.64 among cases). Analysis of the mean number of people in the house by income ranges showed that the mean number of people in the house was highest for the middle-income range. The odds of being a case were not significantly higher amongst those that lived in a flat compared with those that lived in a house and amongst those that rented their accommodation compared with those that owned their homes.

Table 4.9: Details about residence, status of accommodation, and plot size of the cases and controls

Residential details	Cases %	Controls %	MOR (95% C.I.)
Residential site			
House	90.5	87.0	1
Flat	9.5	13.0	0.70 (0.37, 1.32)
Accommodation owned or rented			
Owned	63.0	60.0	1
Rented	37.0	40.0	0.88 (0.58, 1.32)
Plot size			
Less than 500 sq. meters	33.9	25.5	2.69 (1.28, 5.25)
500-1000 sq. meters	34.5	31.9	1.86 (1.02, 3.42)
more than 1000 sq. meters	31.6	43.6	1
n	171	194	

The odds of being a case were significantly higher amongst those who lived on plots less than 500 sq. meters in size (p-value=0.003) and amongst those who lived on plots less than 1000

sq. meters in size (p-value=0.04) compared with those who lived on plots larger than 1000 sq. meter in size.

Table 4.10: Ownership rate of articles of household and personal use

Ownership of articles of use	Cases %	Controls %	MOR (95% C.I.)
Do not have Car	62.0	51.5	1.53 (0.98, 2.39)
Do not have Bicycle	63.2	58.8	1.07 (0.71, 1.60)
Do not have Scooter	73.1	73.1	0.97 (0.62, 1.52)
Do not have Television	8.2	9.8	0.71 (0.34, 1.48)
Do not have VCR	59.1	51.5	1.34 (0.88, 2.04)
Do not have Cable network	76.6	70.6	1.44 (0.88, 2.36)
Do not have Air Conditioner	69.6	58.2	1.70 (1.06, 2.74)
Do not have Refrigerator	15.2	17.1	0.82 (0.44, 1.53)
Do not have Telephone	31.6	30.4	1.02 (0.66, 1.58)
Do not have Mobile telephone	88.9	85.6	1.24 (0.65, 2.34)
Do not have Computer	89.5	77.8	2.46 (1.29, 4.69)
Do not have Cattle	79.5	73.2	1.31 (0.78, 2.18)
Do not have Tractor	92.4	91.7	1.00 (0.45, 2.23)
n	171	194	

Ownership of items of household and personal use was also ascertained amongst the study participants; no significant differences were observed between cases and controls.

4.5.2 Discussion

Social position may influence health via pathways mediated by psychosocial factors or independent of them. In this study, accommodation, income and ownership of household and personal items were used as parameters, for assessment of the socio-economic status of the study participants.

Analysis of income by quartile levels showed that the monthly income of 50% of the study participants was less than £116, which is 30% more than the average Pakistani household income of £82.[407] Controls were shown to have higher mean income levels compared with cases. In addition, significantly higher number of cases belonged to the lower income categories compared with controls; caution however, must be exercised in labelling this trend as being indicative of a higher prevalence of CAD amongst the lower socio-economic classes as this may have been influenced by better opportunities that controls had in accessing care. Socio-economic status is also difficult to define and categorize and drawing inferences based on categorization by income levels may not be an entirely true representation of the picture.

In addition to income, social class of the cases and the controls was also indirectly inferred from the ownership of various items of household and personal use. The comparison serves to show differences between the study participants and the general population of the country. The results tabulated in Table 4.11 indicate that the study participants were more affluent than the general population. More than 90% of the cases and controls owned a television, more than 38% in both categories owned a car whereas about 70% of the participants owned a telephone; this contrasts with the ownership rate of 42%, 8.9% and 13% for television, car and telephone in Pakistan respectively and serves to reinforce the earlier impression that the

study participants belonged to a higher socio-economic class compared with the general population of the country. High ownership rate for television at 91.8% for the cases and 90.2 % for the controls (representative of the middle and high socio-economic class) and 42% for the general population has important implications for health promotion initiatives that focus on information dissemination to step up preventive approaches.

Table 4.11: Ownership rate of articles of household and personal use in the study participants compared with the general population of Pakistan

Possession of articles of use	Cases %	Controls %	Population of Pakistan[408]
Car	38.0	48.0	8.9
Bicycle	37.4	46.0	42.0
Scooter	26.9	26.9	16.0
Television	91.8	90.2	42.0
VCR	45.0	48.0	21.0
Cable network	23.0	30.0	-
Air conditioner	31.0	41.0	-
Refrigerator	85.5	82.3	41.0
Telephone	69.0	69.0	13.0
Mobile telephone	11.6	14.0	2.3
Computer	10.5	22.0	1.5
Cattle	20.5	27.0	-
Tractor	7.6	8.2	5.7
n	171	194	

More than 90% of the study participants lived in houses. Whereas living in a house reflects a higher socio-economic status of an individual in the urban western setting, it is not indicative of the same in the Pakistani context. Residential flats are uncommon in the twin cities of Rawalpindi and Islamabad. Plot size of the house, on the other hand, is a better indicator of socio-economic status. An inverse relationship between socio-economic status and conventional risk factors is well known and has been documented for the Caucasian population.[178] In our study, the odds of being a case were found to be significantly higher in those that lived on smaller plots compared with those that lived in houses built on larger plots. Within the study participants, those in lower income categories were also shown to have less leisure time physical activity, consumed more fat, and underwent higher levels of stress compared with those in the higher income categories. There were, however, no differences observed between the mean cholesterol, glucose, LDL and triglycerides levels and systolic and diastolic blood pressure values between groups with different income levels. This trend is clearly one of concurrence of lifestyle risk factors, which includes increased fat consumption, stress and physical inactivity amongst the lower socio-economic classes; concurrence of risk factors is known to have a synergistic effect on the risk for CVD. These risk factors may be labelled as "remote" in comparison with the biological risk states, which may be termed as "proximate" for which no differences existed between groups in different income levels. This is in contrast with reported findings in the Caucasian population.

In the Caucasian population, an inverse relation between SES and several biological, behavioural, psychological and social risk factors including hypertension, smoking, total cholesterol, BMI, diabetes, and LDL have been documented. These risk factors do contribute to but do not fully explain the cardiovascular health inequalities among socio-economic groups.[409,410] This suggests that there are other neuro-endocrine and physiologic pathways in the mediation of socio-economic inequalities in CAD risk. It needs to be determined whether

the same holds true for the Pakistani population. The first step in this approach would be to identify a method for the valid categorization of socio-economic groups in this population, followed by the determination of the prevalence of CAD and its risk factors in various socio-economic groups. It would then, be possible to ascertain, whether there are any socio-economic gradients in coronary risk in this population and if so, to what extent they can be explained on the basis of conventional risk factors. This exercise has important implications, as it will help to identify risk characteristics that could be the target of public health interventions to modify risk factors in disadvantaged communities. This data gives useful preliminary insight into the issue by identifying adverse lifestyle characteristics amongst the poorer Pakistani communities.

4.6 EDUCATION

4.6.1 Results

8.5% of the participants were uneducated, 10.8% had less than 5 years of schooling, 29.6% had 5-10 years of schooling, and 30.3% had between 10-14 years of education whereas 20.3% had been educated for 14 years or more. No significance difference was seen in the mean duration, for which the cases (mean 10.98, SE=0.33) and controls (mean 10.40, SE=0.36) had been educated (p-value=0.23).

Table 4.12: Distribution of risk factors in categories with different level of education

	Schooling					
	No education	Below grade 5	Grade 5-10	Grade 10-14	Over grade 14	All
Activities while awake						
Sedentary	48.4	37.8	34.8	38.1	31.3	36.7
Light activity	35.5	45.9	44.6	37.2	52.2	43.1
Heavy physical activity	16.1	16.2	20.5	24.8	16.4	20.3
Exercise*						
Useful	11.8	37.2	44.1	61.2	61.7	49.4
Not useful	88.2	62.8	55.9	38.8	38.3	50.6
Smoking						
Never smoked nor passive smoker	21.2	11.1	6.6	9.1	6.6	9.2
Ever smoker but not a passive smoker	9.1	2.8	5.7	4.5	1.6	4.6
Never smoked but passive smoker	42.4	36.1	31.1	39.1	47.5	38.2
Ever smoker as well as passive smoker	27.3	50.0	56.6	47.3	44.3	48.0
n	34	43	118	121	81	397

* p-value =<0.001

Analysis of the risk factor prevalence in individuals with varying levels of education yielded significant results for exercise only; a significant trend for useful exercise was observed

amongst those who were better educated (χ^2=36.0; p-value=<0.001). There were no differences observed in the status of smoking (χ^2=2.67; p-value=0.84), daily activities (χ^2=2.48; p-value=0.77), visible fat consumption (χ^2=4.58; p-value=0.46) and the occurrence of high blood pressure (χ^2=0.60; p-value=0.73) and diabetes (χ^2=4.64; p-value=0.09) amongst individuals with different levels of education.

There were likewise no significant differences observed when mean cholesterol (F=0.91; p-value=0.48, One Way ANOVA), diastolic (F=1.6; p-value=0.37, One Way ANOVA) and systolic blood pressure levels (F=1.78; p-value=0.11, One Way ANOVA), glucose (F=0.86; p-value=0.50, One Way ANOVA) and BMI (F=0.36; p-value=0.90, One Way ANOVA) were compared between study participants with different levels of education.

4.6.2 Discussion

Inverse associations between educational level and the prevalence of smoking, physical inactivity, obesity, hypercholesterolaemia, low HDL levels and hypertension have been demonstrated in the developed world setting; in addition, concurrence of cardiovascular risk factors is also known to occur more frequently in the lower, than in the higher educated groups. Studies have also observed that most of the associations between risk factors do not depend on educational level; this strongly suggests that the higher concurrence of risk factors among lower educational level can only be attributed to the higher prevalence of individual risk factors.[179] These independent inverse associations between education and smoking, blood pressure and risk of mortality, over and above the impact of known risk factors, suggest that socio-economic status has a complex impact on health.[411] In this study, however, study participants were a homogenous group with regard to the level of education, with majority of the participants being educated up to tenth grade; differences in the level of education were not wide enough to reveal meaningful differences in the risk factor distribution and therefore differences in risk factor prevalence between educational categories have been insignificant.

4.7 ETHNIC IDENTITY

4.7.1 Results

Cases and controls in this study were not matched for ethnic identity. 64.7% of the participants were *Punjabi*, 16% were *Pathans*, 0.3% were *Balochis,* 1% were *Sindhis*, 8.5% were *Mohajirs,* and 7.8% were *Kashmiris* while 1.8% were of minority ethnic origin. A breakdown of ethnic identities in cases and controls is tabulated; in addition, associations of different ethnic identities with risk factors have also been determined.

Table 4.13: Ethnic identities of the cases and controls

Ethnic identity	Controls %	Cases %
Punjabi	62.0	67.0
Pathan	16.5	15.5
Mohajir	8.5	8.5
Kashmiri	9.5	6.0
Other	3.5	3.0
n	200	200

Table 4.14: Risk factor distribution in different ethnic identities

	Ethnic Identity				
	Punjabi %	Pathan %	Mohajir %	Kashmiri %	Other %
Exercise					
Useful	49.6	48.4	58.8	41.9	46.2
Not useful	50.4	51.6	41.2	58.1	53.8
Blood Pressure					
Normal	33.1	32.8	30.3	42.3	36.4
High	66.9	67.2	69.7	57.7	63.6
Smoking status					
Current smoker	19.1	8.2	12.1	29.0	0.0
Past smoker	34.1	45.9	42.4	41.9	38.5
Never smoker	46.7	45.9	45.5	29.0	61.5
Activities while awake					
Sedentary	36.7	37.7	32.3	40.0	33.3
Light activity	44.1	41.0	38.7	43.3	41.7
Heavy activity	19.2	21.3	29.0	16.7	25.0
Family history of disease[iv]					
No	56.0	39.7	35.3	45.2	46.2
Yes	44.0	60.3	64.7	54.8	53.8
Mean BMI (SE)	25.34 (0.22)	26.56 (0.55)	24.74 (0.68)	24.55 (0.56)	25.28 (0.80)
Mean waist-hip- ratio (SE)	0.92 (0.005)	0.94 (0.008)	0.91 (0.01)	0.93 (0.001)	0.89 (0.017)
Mean age (SE)	49.4 (0.59)	50.28(1.26)	51.62 (1.77)	49.7(1.59)	48.7 (3.25)

Table 4.15 Mean levels of biochemical risk markers in different ethnic identities

Biochemical parameters	Ethnic Identity				
	Punjabi	Pathan	Mohajir	Kashmiri	Others
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Cholesterol	4.34 (0.07)	4.23 (0.14)	4.18 (0.19)	3.9 (0.21)	4.1 (0.19)
HDL	0.78 (0.01)	0.81 (0.28)	0.90 (0.44)	0.73 (0.37)	0.83 (0.58)
LDL	2.88 (0.01)	2.75 (0.12)	2.71 (0.16)	2.61 (0.17)	2.52 (0.04)
Apo A1	1.14 (0.12)	1.14 (0.03)	1.21 (0.04)	1.06 (0.03)	1.19 (0.05)
Apo B	0.92 (0.01)	0.90 (0.03)	0.87 (0.04)	0.92 (0.05)	0.92 (0.05)
Triglycerides	1.41 (0.04)	1.41 (0.09)	1.19 (0.08)	1.26 (0.10)	1.6 (0.16)
Lp(a) (log)	-2.8 (0.06)	-2.9 (0.11)	-2.49 (0.16)	-2.77 (0.19)	-2.5 (0.24)
Glucose	6.47 (0.16)	6.40 (0.23)	7.05 (0.46)	5.99 (0.34)	5.71 (0.66)
Insulin	33.1 (3.64)	45.8 (8.24)	50.3 (14.9)	25.1 (9.04)	62.8 (23.4)
Insulin resistance	11.5 (1.59)	15.68 (3.37)	20.1 (6.28)	9.83 (5.53)	7.51 (1.85)
Fibrinogen	2.31 (0.07)	2.58 (0.31)	2.51 (0.24)	2.29 (0.23)	2.21 (0.18)
Parathyroid hormone	69.15 (3.06)	61.02 (3.32)	69.7 (4.28)	57.68 (3.49)	60.1 (6.39)
Albumin	49.2 (0.24)	48.6 (0.43)	49.4 (0.58)	48.3 (0.52)	50.0 (1.08)
ALT	18.0 (2.54)	22.7 (3.92)	19.0 (3.30)	19.7 (4.25)	18.2 (2.26)

[iv] Hypertension, diabetes, stroke, sudden non accidental death and coronary heart disease.

Continued.....

Biochemical parameters	Ethnic Identity				
	Punjabi	Pathan	Mohajir	Kashmiri	Others
AST	25.6 (0.94)	28.4 (2.29)	26.3 (2.84)	25.7 (2.5)	28.6 (4.02)
Sialic acid	80.9 (0.94)	79.6 (1.67)	78.0 (1.91)	74.4 (2.75)	78.1 (3.13)
CRP (log)	1.72 (0.08)	1.93 (0.17)	1.47 (0.25)	1.53 (0.20)	1.42 (0.34)
Protein	86.5 (0.86)	84.7 (2.21)	85.9 (2.12)	79.4 (1.88)	87.3 (3.30)
Creatinine	97.4 (1.98)	99.87 (3.17)	100.57 (4.39)	103.21 (5.2)	87.1 (8.18)
Bilirubin	6.19 (0.32)	7.02 (0.57)	6.11 (0.49)	6.91 (0.62)	5.97 (1.03)
Creatine kinase	87.9 (5.78)	78.5 (10.4)	92.6 (15.3)	113.1 (17.7)	132.5 (47.9)
Gamma GT	29.9 (1.34)	38.98 (7.84)	31.3 (4.17)	30.5 (5.41)	22.0 (3.12)

Associations of different ethnic identities with risk factors levels revealed significant differences in BMI (p-value=0.06) and family history (p-value=0.04); *Pathans* had a significantly higher BMI compared with the others whereas *Punjabis* were found to have a more significant association with family history.

4.7.2 Discussion

There are five major ethnic groups living within Pakistan; they can be identified from their native province of residence. People from the eastern province of Pakistan and the north-western part of India are called *Punjabis*; the region has been invaded by many different races including the *Aryans, Persians,* and the *Mongols*. The racial and cultural characteristics of this population therefore reflect this diversity as well as the rich cultural heritage of one of the earliest civilizations dating back to 3000 B.C. *Kashmiris* hail from the north-eastern part of Pakistan. The term *Kashmiri* is a wide term loosely applied to several streams of immigrants from Turkey, Iran, Central Asia and Afghanistan who settled in the valley of Kashmir. The Indo-Aryans and Indo-Greeks have significantly influenced the racial composition, religion and language of the *Kashmiris*.

The *Pashtuns* or the *Pathans* constitute the majority of the population of the North West Frontier Province and also some parts of the province of Balochistan. They arose from an intermingling of the ancient Aryan army from the north and the west with subsequent invaders. Several *Pashtun* clans are known to have moved from Afghanistan to Pakistan between the 13 to the 16th century. *Sindhis,* on the other hand, belong to the province of Sindh in the lower Indus valley, home to the Indus valley civilizations dating back to 3000 B.C. People of the south-western province of Pakistan, Balochistan are called *Balochis*; these are mostly pastoral tribes chiefly of *Barohi, Baluch*, and *Pathan* origin. The word *Mohajir* means immigrant; *Mohajirs* are the Muslim population, which migrated from India to Pakistan during the partition of India in 1947 and therefore constitute a diverse population from all parts of India and Bangladesh.

Whereas these different ethnic groups are seen to have distinct origins, there is nevertheless, significant overlapping and intermingling over the years. There has never been an attempt at comparing the coronary risk status of the different ethnic groups within Pakistan and the absence of hospital-based data from various provinces makes it impossible to compare the baseline risk characteristics of this population. In this study, significant differences were not shown for the various risk factors between groups with different ethnic identities. Differences

would be important to explore with regard to their implications for preventive strategies within different ethnic groups; these differences should be further explored in population-based studies with groups represented by larger sample sizes.

4.8 PAST HISTORY

4.8.1 Results

4.8.1.i Hypertension and diabetes

A significant association of diabetes and high blood pressure was found with disease. The odds of being a case were found to be significantly higher in those with both diabetes and high blood pressure (MOR 3.92; 95% C.I. 1.83, 8.36; p-value=<0.001) and amongst those with diabetes (MOR 3.35; 95% C.I. 1.26, 8.88; p-value=0.01) compared with controls. However, the odds of being a case was not found to be significantly higher amongst those that suffered from high blood pressure only (MOR 1.33; 95% C.I. 0.82, 2.15; p-value=0.25).

Table 4.16: Self-reported past history of diabetes and high blood pressure in cases and controls

Past history of diabetes and high blood pressure	Case %	Control %	MOR (95% C.I.)
No history	41.5	55.4	1
History of diabetes	9.3	4.7	3.35 (1.26, 8.88)
History of high blood pressure Diabetes	31.1	33.2	1.33 (0.82, 2.15)
and high blood pressure	18.1	6.7	3.92 (1.83, 8.36)
n	193	193	

4.8.1.ii Duration of high blood pressure: no significant difference was found between the mean duration of years for which the cases (7.44; SE=0.78) and controls (7.27; SE=0.79) suffered from high blood pressure (MOR 1.005; 95% C.I. 0.94, 1.08; p-value=0.88).

4.8.1.iii Duration of diabetes: likewise, there was no difference observed between the mean duration of years for which the cases (8.33; SE=0.89) and controls (7.29; SE=1.39) suffered from diabetes (MOR 1.001; 95% C.I. 0.89, 1.13; p-value=0.98).

4.8.1.iv Stroke: 7% (14/200) cases and 2.5% (5/200) controls gave a history compatible with a stroke in the past. The odds of having had a stroke in the past were significantly higher amongst cases compared with controls. (MOR 3.25; 95% C.I. 1.06, 9.97; p-value=0.02).

4.8.2 Discussion

Hypertension and diabetes are well known biological risk states for CAD. Diabetes in particular, is of great concern to South Asian countries. Therefore analysis of the past history of the cases and controls yielded expected results; more cases compared with controls suffered from high blood pressure and diabetes. The association was stronger for the presence of concomitant high blood pressure and diabetes. There was however, no difference

observed in the mean systolic and diastolic blood pressure levels between the cases and the controls; this could be explained on the basis of them being under treatment for these diseases. More cases in comparison to controls, had suffered from stroke in the past, which was indicative of the higher prevalence of atherosclerotic disease in the cases.

Demonstration of the strong association of hypertension and diabetes with the risk of CAD in this population has important implications, reinforcing that these are equally important risk states in the Pakistani population. Diabetes and hypertension form the cornerstone of the high risk population strategy in CVD prevention; both these biological risk states serve as cut off points in the public health approach to prevention at the population level, separating individuals that need to be dealt with a more aggressive risk reduction approach.

4.9 SMOKING

4.9.1 Results

4.9.1.i Cigarette smoking: status of smoking: more than 50% of the study participants either smoked currently or had been smoking in the past. The odds of being a case was significantly higher, both amongst those that smoked currently (p-value=0.03) and amongst those that smoked in the past (p-value=0.03).

Table 4.17 Current and past smoking status of cases and controls

Smoking status	Case %	Control %	MOR (95% C.I.)
Current smoker	19.0	14.8	1.88 (1.02, 3.49)
Past smoker	41.0	33.9	1.88 (1.10, 3.19)
Never been a smoker	40.0	51.3	1
n	195	189	

Type of smoking practiced: amongst the smokers, 94.7% were cigarette smokers, 1.9% were hukka smokers, and 3.4% smoked both the hukka and cigarettes whereas no one reported smoking other forms of tobacco. When the data on smoking cigarettes and hukka was combined, a non-significant dose-response effect was evident. (MOR 1.32; 95% C.I 0.89,1.96).

Number of pack years smoked: cases smoked for significantly higher mean number of pack years (222.76; SE=32.01) compared with controls (122.81; SE=16.39). The difference was found to be significant (MOR 1.001; 95% C.I. 1.00, 1.002; p-value=0.001).

Number of cigarettes smoked: cases were also seen to smoke a higher number of cigarettes (18.22; SE=1.24) compared with controls (14.0; SE=1.08) and therefore the odds of being a case were significantly higher amongst those that smoked a higher number of cigarettes (MOR 1.3; 95% C.I. 1.2,1.06; p-value=0.04).

4.9.1.ii Passive Smoking

Frequency of passive smoking: 82% of the controls and 90.6% of the cases were exposed to environmental tobacco smoke on a regular basis. Cases had significantly higher odds of exposure to passive smoking compared with controls (MOR 2.87, 95% C.1. 1.28, 6.42; p-value=0.01) when no passive smoking was taken as a reference category. However, when no active or passive smoking was taken as a reference category, only those with exposure to both had significantly higher odds of being a case (p-value=0.02). This is shown in Table 4.18.

Table 4.18 Exposure to active and passive smoking in cases and controls

Passive smoking or ever-smoker	Case %	Control %	MOR (95% C.I.)
No active or passive smoking exposure	6.0	12.0	1
Active but not a passive smoker	3.6	5.5	0.81 (0.17, 3.79)
No active smoking but passive smoker	36.1	39.9	1.71 (0.65, 4.51)
Active as well as passive smoker	54.2	42.6	3.09 (1.14, 8.39)
n	166	183	

Duration of passive smoking exposure: no significant differences were observed in the mean duration for which cases and controls were exposed to passive smoking. The odds of being a case were not higher amongst those who were exposed to environmental tobacco smoke for more than half an hour compared with those who were exposed for less than half an hour (MOR 1.33; 95% C.I. 0.81, 2.20; p-value=0.25).

Frequency of passive smoking exposure: the odds of being a case were significantly higher amongst those who were exposed to passive smoking on a daily basis (MOR 3.87; 95% C.I. 1.68, 8.86; p value=0.001) when no exposure was taken as a reference category.

Table 4.19 Passive smoking exposure level in cases and controls

Passive smoking exposure level	Case %	Control %	MOR (95% C.I.)
Never exposed	7.6	17.1	1
Exposed less than once a week	10.0	13.5	2.16 (0.81, 5.81)
One to two times a week	15.3	14.5	2.95 (0.95, 6.42)
Three to four times a week	5.3	8.8	1.55 (0.54, 4.53)
Daily exposure	61.8	46.1	3.87 (1.68, 8.86)
n	170	193	

Source of passive smoking: a detailed history was sought to ascertain the source of passive smoking exposure. Results are summarized in Table 4.20. Cases were seen to have significantly higher odds of exposure to environmental tobacco smoke as a results of spousal smoking (p-value=0.04).

Table 4.20 Source of passive smoking exposure in cases and controls

Smoking by	Case %	Control %	MOR (95% C.I.)
Spouse	11.8	4.1	2.38 (1.04, 5.42)
Parents	20.0	18.1	1.20 (0.66, 2.17)
Friends	60.8	54.9	1.36 (0.81, 2.28)
Co-workers	49.7	45.6	1.27 (0.76, 2.12)
Children	2.9	2.6	1.00 (0.25, 4.00)
Siblings	28.1	26.4	1.17 (0.71, 1.92)
Others	28.1	24.9	1.24 (0.76, 2.02)
n	171	193	

4.9.1.iii Smokeless tobacco: 8.3% (16/192) of the controls and 9.4% (18/192) of the cases used oral chewable tobacco. The difference was not found to be significant. (MOR 1.14; 95% C.I. 0.56, 2.34; p-value=0.71).

4.9.2 Discussion

The relationship between smoking and CAD has been well established and is beyond discussion and debate. Current smokers have a 70% increased risk of fatal CHD.[22] In this study, the odds of CAD amongst current and past smokers were significantly higher compared with those who did not smoke; the observed odds ratios were however lower than expected. Part of the explanation for this trend could stem from the study design itself. Cases and controls in this study were both drawn from a study population that had been referred for coronary angiography; referral for this investigation requires a strong index of suspicion for underlying CAD and the decision is based not only on the symptomatic severity of the disease but also on a balanced assessment of the coronary risk profile of the individual; an adverse coronary risk profile is therefore a source of referral bias. For this reason, the inclusion of controls with, on the average, a more adverse coronary risk profile than the general population failed to show the expected level of differences in significance in the current or past status of smoking between the cases and controls.

There were however, other significant differences, which also highlighted the greater exposure of cases to tobacco compared with controls. Cases were shown to smoke for a significantly longer duration and significantly larger number of cigarettes as compared to the controls. This observation gives valuable insight into the higher level of exposure of the cases to tobacco compared with controls. The current emphasis on smoking cessation in Pakistan is therefore very relevant to these findings and needs to be further strengthened.

The public health impact of exposure to the tobacco smoke of others is considerable. Epidemiological studies have shown an increased risk of about 20% for CAD for those who have never smoked but are exposed to environmental tobacco smoke. Almost all of these studies showed an increased risk of heart disease for the exposed groups (usually defined as currently exposed) compared with the non-exposed group. A number of studies controlled for the principal heart disease risk factors, and several showed a positive dose response trend according to the level of presumed environmental tobacco smoke exposure.[34]

In this study, cases exposed to passive smoking on a daily basis had significantly higher odds of CAD compared with those with no such exposure. Passive smoking as a result of the spouse smoking was identified as being more significant amongst the cases. Spousal exposure has been found to be an important source of environmental tobacco smoke. In a meta analysis on passive smoking,[412] most of the studies were based primarily on analysis of spousal smoking; the relative risk of 1.23 (95% C.I. 1.12,1.35) for heart disease mortality (1.23 for women and 1.25 for men) for environmental tobacco smoke exposure for a spouse among never-smokers was found. This was also confirmed in the largest prospective study to date involving more than 300,000 women followed up for over 7 years in which analysis focused on sub cohorts of 309,599 married pairs and 135,237 subjects concordant for self reported exposure and exposure reported by each ones' spouse. In this study, after controlling

for many risk factors, a 22% higher CHD mortality (rate ratio 1.22; 95% C.I. 1.07,1.40) was found among never-smoking men married to currently smoking wives compared with those married to wives who had never smoked. The corresponding rate ration for women was 1.10 (0.96,1.27). Never-smokers living with former smokers showed no such increase further reinforcing the role of spousal passive smoking.[34]

In another study,[413] however, no increase in CAD risk was found due to environmental tobacco exposure from a spouse. This may have been due to using "any" spousal exposure to environmental tobacco smoke, which dilutes the effects amongst current smokers by including former smokers. They did not report current as a separate single category.

These findings indicate that consideration must be given to passive smoking exposure in the assessment of cardiovascular risk not only in the primary and secondary prevention settings in individual patients but also as part of public health initiatives.

Smokeless oral tobacco is a major issue in Pakistan, it is taken both in the form of *naswar* or snuff and in the form of *pan*. 10% of the Pakistani population uses it in one form or the other and the stigma associated with smoking a cigarette has increased its use in females in Pakistan. It has been shown that the use of smokeless tobacco, during which nicotine is absorbed through the buccal mucosa, produces maximum blood levels of nicotine similar to those produced by cigarette smoking.[414,415] It is therefore, not surprising that the risk of dying from cardiovascular disease is higher amongst those that use smokeless tobacco compared with those who do not. In this study, more cases compared with controls used smokeless tobacco. The difference however was found to be insignificant.

4.10 DIET

4.10.1 Results

4.10.1.i Portions of food items per week: number of servings of various food items consumed on a weekly basis has been shown in Table 4.21. For each food item, a reference category has been defined and odds ratios computed.

Table 4.21: Servings per week of various food items consumed by the cases and controls with matched odds ratio compared to the category with least risk

Food items	Case %	Control %	MOR (95% C.I.)
Beef			
Less than once a week	29.0	31.0	1
1-2 times a week	38.0	36.0	1.14 (0.70, 1.87)
3-5 times a week	26.5	26.5	1.08 (0.65, 1.8)
6 or more times a week	6.5	6.5	1.07 (0.46, 2.45)
Lamb Mutton			
Less than once a week	33.0	31.5	1
1-2 times a week	30.5	37.5	0.71 (0.41, 1.12)
3-5 times a week	28.5	23.0	1.120 (0.67, 1.88)
6 or more times a week	8.0	8.5	0.87 (0.37, 1.99)
Chicken			
Less than once a week	27.5	22.5	1
1-2 times a week	48.0	49.0	0.9 (0.53, 1.30)
3-5 times a week	23.0	25.0	0.74 (0.42, 1.3)
6 or more times a week	1.5	3.5	0.27 (0.55, 1.41)
Fish			
Never	16.5	29.0	0.33 (0.09, 1.15)
Once a week	68.0	54.0	0.70 (0.20, 2.39)
1-2 times a week	11.0	14.5	0.43 (0.11, 1.65)
3 or more times a week	4.5	2.5	1
Vegetable curry			
Never/Once a week	2.5	2.0	2.24 (0.57, 8.90)
1-2 times a week	20.5	17.0	2.17 (1.16, 4.04)
3-5 times a week	56.0	44.5	2.09 (1.32, 3.32)
6 or more times a week	21.0	36.5	1
Fried foods			
Never	7.5	15.5	1
Once a week	45.0	34.0	3.00 (1.38, 6.51)
1-2 times a week	25.5	24.5	2.33 (1.04, 5.21)
3-5 times a week	15.5	13.0	2.49 (1.03, 6.04)
6 or more times a week	6.5	13.0	1.24 (0.46, 3.33)
Cooked breakfast			
Never	51.5	57.5	1
Once a week	1.0	3.0	0.41 (0.08, 2.05)
1-2 times a week	6.5	7.0	1.07 (0.49, 2.36)
3-5 times a week	12.5	14.5	0.98 (0.54, 1.79)
6 or more times a week	28.5	18.0	1.79 (1.07, 2.98)
Dal (lentils)			
Less than once a week	5.0	7.5	0.83 (0.35, 1.96)
1-2 times a week	61.5	45.0	1.99 (1.29, 3.01)
3 or more times a week	33.5	47.5	1
Sweets			
Never	5.5	7.5	1
Once a week	38.5	29.0	1.94 (0.81, 4.66)
1-2 times a week	32.5	27.0	1.83 (0.73, 4.59)
3-5 times a week	18.5	28.5	0.98 (0.39, 2.44)
6 or more times a week	5.0	8.0	0.88 (0.31, 2.55)
Jam			
Never	51.0	55.9	1
Once a week	22.0	16.5	1.54 (0.88, 2.68)
1-2 times a week	12.5	10.1	1.29 (0.66, 2.52)
3-5 times a week	7.5	9.0	0.91 (0.42, 1.97)
6 or more times a week	7.0	8.5	1.03 (0.47, 2.25)

Continued.....

Food items	Case %	Control %	MOR (95% C.I.)
Honey			
Never	47.5	47.3	0.74 (0.31, 1.80)
Once a week	28.5	29.0	0.72 (0.29, 1.81)
1-2 times a week	10.5	10.2	0.70 (0.25, 1.98)
3-5 times a week	6.0	8.1	0.47 (0.15, 1.50)
6 or more times a week	7.5	5.4	1
Fibre			
1-2 times a week	2.0	4.0	0.43 (0.13, 1.46)
3-5 times a week	27.0	35.5	0.66 (0.43, 1.01)
6 or more times a week	71.0	60.5	1
Rice			
Less than once a week	21.6	28.9	0.70 (0.41, 1.2)
1-2 times a week	45.7	42.1	0.96 (0.60, 1.5)
More than 3 times a week	32.7	28.9	1
Fruit			
Never	1.0	2.0	0.53 (0.09, 3.04)
Once a week	10.0	6.5	1.59 (0.65, 3.91)
1-2 times a week	17.0	19.5	0.80 (0.46, 1.39)
3-5 times a week	26.5	30.5	0.78 (0.48, 1.28)
6 or more times a week	45.5	41.5	1
Beans and peas			
Less than once a week	33.5	25.0	3.15 (1.72, 5.7)
1-2 times a week	51.5	40.5	2.8 (1.66, 4.8)
More than 3 times a week	15.0	34.5	1
Potatoes			
Once a week	13.5	13.6	1.16 (0.61, 2.18)
1-2 times a week	45.5	34.8	1.66 (1.07, 2.59)
3 or more times a week	41.0	51.5	1
Raw vegetables			
Once a week	10.9	16.1	0.57 (0.30, 1.08)
1-2 times a week	15.6	16.7	0.89 (0.46, 1.06)
3 or more times a week	73.4	67.2	1
Cereals			
Never	81.5	77.5	0.92 (0.28, 3.08)
Once a week	7.5	9.0	0.65 (0.15, 2.78)
1-2 times a week	6.5	7.5	0.71 (0.18, 2.92)
3-5 times a week	1.5	3.5	0.35 (0.06, 2.02)
6 or more times a week	3.0	2.5	1
Sago grain			
Never	86.0	82.9	0.94 (0.53, 1.67)
Less than once a week	12.5	13.1	0.37 (0.1, 1.41)
Once or more times a week	1.5	4.0	1

The odds of being a case were significantly higher amongst those taking less vegetable curry compared with those taking more than six servings of vegetable curry a week. The odds of being a case were also significantly higher amongst those that took increasing servings of fried foods compared with those that did not take any fried foods. Similarly, the odds of being a case were also significantly higher amongst those that took six or more servings of cooked breakfast a week compared with those that never ate cooked breakfast and amongst those that took fewer servings of lentils compared with those who consumed more than 3 servings of lentils a week.

4.10.1.ii Type of fats used for cooking

Table 4.22: Types of fat used for cooking curries and other forms of cooked food

Type of fat used for cooking	Case %	Control %	MOR (95% C.I.)
Vegetable oil	85.0	79.0	1
Vegetable *ghee*	10.0	11.5	0.80 (0.48, 1.66)
Desi ghee	1.0	1.5	0.65 (.2, 3.92)
Desi ghee plus vegetable oil	0.5	1.0	0.50 (0.04, 5.01)
Vegetable *ghee* plus vegetable oil	3.0	6.5	0.46 (0.17, 1.21)
N	200	200	

No significant differences were observed in the intakes of the types of fats used for cooking by the cases and controls. This has been shown in Table 4.22; the odds of having transferred from *ghee* to oil for cooking were higher amongst cases (MOR 2.06; 95% C.I. 1.15, 3.68; p-value=0.01) compared with controls. Controls, on the other hand, were seen to be using oil rather than *ghee* for a longer duration of time (mean 8.4; SE 0.59) than the cases (7.68; SE 0.49). The difference however was not found to be significant (MOR 0.98; 95% C.I. 0.95, 1.02; p-value=0.53).

4.10.1.iii Eggs

Table 4.23: Consumption of eggs per week by the cases and controls

Intake of eggs	Case Mean (SE)	Control Mean (SE)	MOR (95% C.I.)
Consumption of whole eggs	3.92 (0.23)	3.95 (0.26)	0.99 (0.94, 1.06)
Consumption of egg whites	0.72 (0.13)	0.43 (0.11)	1.11 (0.98, 1.26)
n	200	200	

No significant difference was observed in the quantity of whole eggs (p-value=0.93) and egg whites (p-value=0.09) consumed by the cases and controls.

4.10.1.iv Quantity of visible fat used

Table 4.24: Visible fat consumption per day

Quantity of fat used in a day	Case %	Control %	MOR (95% C.I.)
One tablespoon	33.8	31.5	1
Two tablespoon	39.0	35.0	0.99 (0.61, 1.62)
Three tablespoon	11.3	18.3	0.59 (0.32, 1.10)
Four tablespoon	7.7	10.2	0.68 (0.33, 1.40)
Five tablespoon	3.1	3.0	1.14 (0.33, 3.94)
Six or more tablespoon	5.1	2.0	2.18 (0.66, 7.17)
n	195	197	

There was an increasing trend of disease observed amongst those with a higher consumption of fat in a day; this trend was not found to be significant (p-value=0.20).

4.10.1.v Salt use

Table 4.25: Consumption of added salt at the table

Added salt	Case %	Control %	MOR (95% C.I.)
No	67.2	67.2	1
Yes	32.8	32.8	0.98 (0.64, 1.50)
n	200	200	

The odds of being a case were not significantly higher amongst those who added salt to their meals (p-value=0.91).

4.10.2 Discussion

Very little is known about long-term nutrient and fat intake in the Pakistani population. All methods for measuring such intakes have inherent difficulties due to a lack of a "gold" standard but careful development from several methods give reasonable validity; assessment of the dietary pattern, quantity and composition is important as it provides potential targets for informed CAD prevention.

One method is the 7-day weighed record method. While apparently precise, this method is frequently invalid and underreports. It restricts intakes and may well miss the recording of vital savoury snack foods (e.g. *samosas, pakoras* and other fried snacks which are energy and fat dense) that are consumed when the subject goes visiting or entertaining, a feature of the Pakistani community lifestyle. Such technical difficulties emphasize the importance of using other methods. These include Food Diaries, 24-hour dietary recalls and Food Frequency Questionnaires. FFQs, while less precise, give reasonable estimates of habitual quantitative and qualitative nutritional intakes for groups, rather than the few days covered by food diaries and 24-hour dietary recall or weighted records. FFQ has been used with considerable field success in a recently reported very large Pan-European EPIC study[400] and was the method used in this study.

The limitations of a FFQ for the assessment of dietary intakes notwithstanding, in this study, there appeared to be significant differences in the intakes of several dietary components between the cases and controls. Controls were shown to have significantly higher intake of lentils (p-value=0.005), beans (p-value=<0.001), and vegetable curry (p-value=0.02), whereas cases were shown to have higher intakes of fried foods (p-value=0.01) and cooked breakfast (p-value=0.13). There were however, no differences observed in the intake of red meat, fish, raw vegetables, fruits and eggs as would have been expected.

Studies on expatriate South Asians have revealed that they consume less saturated fat, cholesterol and more polyunsaturated fat compared with the British population; however no studies have looked at fat intake in the native Pakistani setting. In this study, a distinction was made between those that consumed oils and those that consumed *ghee* as a form of visible fat used for cooking. Most commercial oils available in Pakistan contain more than 60% polyunsaturates. *Ghee,* on the other hand, is of two types; it may either be of dairy origin or it may be derived from vegetable oils. The commercial form of *ghee* available in Pakistan is predominantly derived from the hydrogenation of vegetable oils and is known as "*banaspati*

ghee"; this form of *ghee* is high in trans fatty acids. On the other hand, *ghee,* derived from dairy sources by the clarification of butter is high in cholesterol oxides; it is usually home made and is known as "*asli ghee*" (real ghee); this form of *ghee* is a rare and expensive commodity and is very infrequently marketed under the trade name of Butter oil and is used only as an occasional treat in some Pakistani households. High intakes of both the trans isomer of fatty acids is known to be significantly associated with the occurrence of CAD.[126,127] In this study, however there were no significant differences observed in the types of fat used for cooking between the cases and controls. There was however, an increasing trend of disease observed amongst those who consumed a higher quantity of visible fat in a day. Based on the results of this study, it was the quantity, more than the type of fat which had an association with CAD; this was further reinforced by the observation that the odds of being a case were higher amongst those with higher intakes of fried foods and with those who consumed curried breakfast more than 6 times a week; both these food items are known to contain higher quantities of fat.

The observed differences in the intakes of lentils, beans, and vegetable curry between the cases and controls were as expected while absence of differences in the intake of red meat, fish, raw vegetables, fruits and eggs were not expected. This may be attributed to the limitation of FFQs in giving reasonable estimates of habitual quantitative and qualitative nutritional intakes. There is therefore, a need to determine the magnitude of benefit through dietary interventions that have proved to be effective in White populations in the Pakistani context; this is likely to have to have important implications for preventive dietary interventions.

4.11 PHYSICAL ACTIVITY

4.11.1 Results

4.11.1.i Useful exercise: more cases compared with controls engaged in useful exercise; the difference however, was not found to be significant (MOR 1.37; 95% C.I. 0.93, 2.03; p-value=0.11)

Table 4.26 Frequency of useful exercise

Useful exercise	Case %	Control %	MOR (95% C.I.)
Yes	53.5	45.5	1
No	46.5	54.5	1.37 (0.93, 2.03)
n	200	200	

4.11.1.ii Duration of exercise: there was no significant difference observed in the mean number of hours for which the cases (1.08; SE=2.24E-02) and the controls (1.12; SE 3.34E-02) exercised (p-value=0.22).

4.11.1.iii Type of exercise: frequencies with which cases and controls performed different forms of exercise are summarized in Table 4.27. Walking was identified to be the predominant form of exercise; more cases compared with controls were shown to walk.

Table 4.27: Type of exercise

Type of exercise	Cases %	Controls %
Walking	82.07	69.0
Stretch exercises	6.6	1.6
Racquet games	0.9	0.8
Isometric exercises	-	0.8
Walking and stretching	5.6	10.0
Walking and jogging	2.8	5.8
Jogging and stretching	0.9	8.3
Other	1.8	2.5
n	106	120

4.11.1.iv Activities while awake: based on the level of physical activity, cases and controls were categorized into those that were predominantly sedentary, those that engaged in light, and those that performed heavy physical activity.

Table 4.28: Daily physical activity

Activities while awake	Case %	Control %	MOR (95% C.I.)
Sedentary	39.5	34.0	0.85 (0.45, 1.61)
Light activity	37.2	48.2	0.62 (0.35, 1.10)
Heavy physical activity	23.3	17.8	1
n	172	191	

4.11.1.v Hours on the feet after the illness: controls (mean 3.00; SE=0.15) spent a significantly longer duration on the feet after the illness compared with cases (mean 1.93; SE=0.12). The difference was found to be significant (p-value=<0.001).

4.11.1.vi Hours on the feet before the illness: similarly, prior to the illness, controls (mean 4.56; SE=0.17) spent a longer duration on the feet compared with cases (mean 4.09; SE=0.16). The difference was found to be significant (MOR 0.91; 95% C.I. 0.83, 1.00; p-value=0.04).

4.11.1.vii Hours of sleep in a day: controls (mean 6.57; SE=0.11) were seen to spend less time sleeping compared with cases (mean=6.99; SE=0.12). The odds of being a case were 1.21 (95% C.I. 1.04,1.41; p-value=0.01) compared with controls for a one hour increased sleep.

4.11.2 Discussion

Habitual physical activity encompasses leisure physical activity, exercise, sports, worksite activity and personal chores and integrates the daily amount of energy expended for activity. An attempt was made to quantitate all these individually for the cases and the controls in the study.

Majority of the controls (61%) and cases (53%) engaged in exercise regularly; however when useful exercise (defined as a minimum of 30 minutes of walk 3 times a week) was taken into account, more cases (53.5%) compared with controls (45.5%) were found to exercise; the difference however, was not found to be significant (p-value=0.11). The average number of hours spent on exercise was higher than one; walking was the commonest form of exercise. In

this study, however, more cases compared with controls practiced useful exercise and similarly, more cases compared with controls were seen to walk. Part of the explanation of this could stem from the timing of the interview; study participants were interviewed when they had full knowledge of their illness and after they had been counselled by their physicians regarding the need to incorporate physical activity and exercise in their daily schedules. This could explain a higher response rate from the cases compared with controls and might reflect the consciously increased level of physical activity by cases in the recent past. Controls however, were shown to be significantly more active than the cases as evidenced by the controls spending a greater number of hours on the feet both before (p-value=0.04) and after the illness (p-value=<0.001) during the day and less time sleeping (p-value=0.01). This provided some evidence about the controls engaging in higher levels of daytime activity. There were however, no significant differences observed between the level of daily physical activity and the duration of leisure time exercise between the cases and the controls.

While interpreting these findings it needs to be recognized that estimating physical activity within populations is imprecise when relying solely on subject reporting in questionnaires and therefore, the quantification and estimation of physical activity in this study using self-reporting has not yielded expected results. In future study settings, it may be useful to compare reported physical activity with quantitatively measured activity; this may be possible through the use of specialized instruments; one such instrument records force due to acceleration from movements of a small ceramic beam, these are validated against oxygen consumption; such instruments have been used successfully in several field settings. The other approach involves developing a scoring system for physical activity, totalling all elements including leisure physical activity, exercise, sports, worksite activity and personal chores. In this study, the intensity and the pace of walk, which was the predominant form of leisure time physical activity was also not ascertained, it was expected that differences would have been observed as even walking at low to moderate pace confers some protection against CAD.[416]

Physical activity offers a cost effective approach to coronary risk modification in poor countries and should be one of the targets of public health interventions to reduce coronary risk within these populations. Physical activity should ideally be at a moderately intense level for 30 minutes to confer protection but favourable metabolic changes are observed with walking and other low to moderate intensity long duration exercise sessions that do not necessarily cause an increase in maximal oxygen intake.[417] Physical activity can therefore be recommended at all ages and at all stages of physical fitness. As the effect of physical activity is known to be graded, higher energy expenditure from leisure time activity or from exercise at higher intensities is likely to generate even more health benefits. Physical activity offers a great opportunity for intervention in developing countries; it should however be encouraged for women in Muslim cultures in an environment and form that respects religious proscriptions; support from Islamic religious leaders is crucial in endorsing the need for more exercise and in encouraging full participation of women in Muslim cultures.

4.12 STRESS

4.12.1 Results

Table 4.29 summarizes frequencies of stressful events during the year preceding the illness, for which, responses were measured on a binary scale. On the other hand, Table 4.30 does the same for stress variables, for which, responses were sought on an ordinal scale.

There were no significant differences observed in the level of stress experienced by the cases and controls as a result of death of the spouse (p-value=0.31), death in the immediate family (p-value=0.51), marital separation (p-value=1.00), history of family conflict (p-value=0.59), loss of crop (p-value=0.41), business failure (p-value=0.27), violence (p-value=0.36) or personal injury (p-value=0.83). Loss of job was the only significant stress factor in this study (p-value=0.02); the odds of being a case were significantly higher amongst those that experienced stress due to loss of job (MOR 3, 95% C.I. 1.09, 8.25).

Table 4.29: Stressful events measured on the binary scale

Stressful events	Cases %	Controls %	MOR (95% C.I.)
Death of spouse	1.8	1.1	3.00 (0.31, 28.84)
Death in a family	43.3	45.3	1.15 (0.75, 1.76)
Marital separation	2.4	2.1	1.00 (0.25, 4.00)
History of divorce	-	1.6	-
History of family conflict	23.4	26.4	0.87 (0.51, 1.46)
Loss of crop	2.3	1.0	2.00 (0.37, 10.92)
Loss of job	9.4	3.1	3.00 (1.09, 8.25)
Business failure	10.5	6.2	1.50 (0.72, 3.11)
Violence	3.5	4.7	0.57 (0.17, 1.95)
Personal injury	7.6	7.3	1.09 (0.48, 2.47)
n	200	200	

Table 4.30: Stressful events in the last one-year measured on the ordinal scale

Stressful factor	Cases %	Controls %	MOR (95% C.I.)
Stress by children			
Never	48.7	52.6	1
Sometimes	34.9	32.1	1.19 (0.75, 1.88)
Several times a week	12.2	10.5	1.20 (0.61, 2.37)
Always	4.2	4.7	0.98 (0.34, 2.83)
Stress by spouse			
Never	56.6	53.6	1
Sometimes	31.2	31.8	0.87 (0.56, 1.37)
Several times a week	9.0	10.4	0.94 (0.46, 1.92)
Always	3.2	4.2	0.70 (0.23, 2.07)
Work stress			
Never	45.1	45.8	1
Sometimes	24.7	25.1	0.78 (0.43, 1.40)
Several times a week	22.2	19.6	1.01 (0.53, 1.91)
Always	8.0	9.5	0.81 (0.35, 1.87)
n	200	200	

Continued.....

Stressful factor	Cases %	Controls %	MOR (95% C.I.)
Stress at home			
Never	43.1	43.3	1
Sometimes	31.6	35.6	1.04 (0.63, 1.71)
Several times a week	18.4	14.4	1.40 (0.76, 2.57)
Always	6.9	6.7	1.27 (0.49, 3.30)
Stress related to daughters' marriages			
Never	63.1	73.6	1
Sometimes	23.1	19.0	1.42 (0.77, 2.62)
Several times a week	10.0	6.1	2.45 (0.96, 6.21)
Always	3.8	1.2	2.64 (0.51, 13.67)
Stress related to in-laws			
Never	86.8	81.3	1
Sometimes	10.5	12.4	0.82 (0.44, 1.52)
Several times a week	1.6	3.1	0.50 (0.12, 2.00)
Always	1.1	3.1	0.33 (0.07, 1.65)
Stress related to in-laws of children			
Never	82.9	92.3	1
Sometimes	12.6	6.2	3.00 (0.81, 11.08)
Several times a week	4.5	1.5	2.00 (0.37, 10.92)
Always	0.0	0.0	-
n	200	200	

No significant associations were found for stressful events measured on the ordinal scale and disease as is evidenced by the following p-values. Stress related to in-laws of children (p-value=0.16), stress related to own in-laws (p-value=0.33), stress related to daughters' marriages (p-value =0.14), stress at home (p-value =0.74), work stress (p-value =0.80), stress by spouse (p-value=0.88) and stress by children (p-value =0.87).

4.12.2 Discussion

Results revealed that cases with a history of loss of job in the year prior to the illness had a 1.7 times higher odds of developing CAD compared with controls; this is indicative of the role of economic issues as the major stress factor in this population. No other significant differences were found between the cases and controls in the type and levels of stresses experienced.

In the developed world populations, psychosocial factors have been documented to contribute significantly to the pathogenesis and expression of CHD; this evidence is composed largely of data relating CAD risk to five specific psychosocial domains: depression, anxiety, personality factors and character traits, social isolation, and chronic life stress. Studies carried out on expatriate South Asians in the UK comparing South Asians to the White population have shown that South Asians suffer from higher levels of depression, higher negative supports, less social support at home, less job control, more effort reward imbalance and higher hostility levels. However, expatriate South Asians may be different from the indigenous South Asian populations because of the influence of lifestyle that accompanies migration to more developed countries; additionally, in the context of stress, the considerations for expatriates may be different with a greater relevance of stresses related to adjustment in the host community. There is therefore a need to define stress factors relevant

to the native Pakistani setting and to determine their association with coronary risk. In this study, the questionnaire was geared to qualitatively analyze stress factors specific to the Pakistani population; these include stresses related to living together in the joint family system and the problems posed in this system by interacting with in-laws, issues relating to the daughters' marriages and economic stresses specific to circumstances in a rural agrarian society such as loss of crop. These stress factors were therefore meant to be indicators of chronic life stress in the Pakistani context. The questionnaire did not however, have assessment components that could have looked at depression, personality traits and social isolation; this was one of the weaknesses of this study.

There are several issues in determining the level of stress. In the first place, the value of self-reported personality factors and character traits is known to be doubtful. Psychological measures used can generate systematic biases in the way individuals answer questions and can result in spurious differences; therefore validation studies are required on existing instruments even for the populations where stress factors have been documented to be associated with coronary risk.

Pakistanis may in addition have a whole host of as yet, poorly identified and measured psychosocial and economic stress factors; cultural changes such as urbanization, break down of family and social support systems, shift from a rural agrarian to a service and manufacturing economy while facing acute demographic and economic pressures are expected to increase both universal and culturally specific CVD stresses in future. There is therefore a need to identify these stresses and to determine their relationship with CAD in this population.

Even in western populations, the underlying pathophysiologic mechanisms through which stress factors affect CVD risk need to be identified fully. The ability of each individual psychological factor, (documented to be associated with coronary risk in the White population) to predict coronary risk within the Pakistani population awaits determination. If psychosocial factors do play a role, then, the biological pathways through which they influence athero-thrombotic and myocardial processes leading to coronary disease await elucidation. Clearly, we are far from establishing these for the Pakistani population where basic answers need to be sought. Understanding of these mechanisms is crucial as it opens doors to the formulation of strategies to modify psychological risk.

4.13 BIRTH WEIGHT

4.13.1 Results

4.31: Self reported birth weight

Birth weight	Case %	Control %	MOR (95% C.I.)
Normal or over weight	79.5	67.7	1
Low birth weight	8.5	8.1	1.12 (0.52, 2.44)
Do not know	12.0	24.2	2.44 (1.38, 4.32)
n	200	198	

The odds of being a case amongst those who were born underweight were not found to be significantly higher compared with those who had normal weight at birth, (MOR 1.12; 95% C.I. 0.52, 2.44; p-value=0.86). Similarly, for those who did not recall their birth weight, the odds of being a case were much higher (MOR 2.44; 95% C.I. 1.38, 4.32; p-value=0.002) compared with those who were born with normal weight.

4.13.2 Discussion

The presence of a significantly higher number of study participants without knowledge of their birth weight generating a very high level of "don't know" response made the meaningful interpretation of this data difficult. Even in the absence of this, subjective evaluation of birth weight, based on distant recall of second hand information is of limited value in the assessment of the relationship of birth weight with CAD. The ideal approach to this is verification of information through hospital records or birth certificates. In Pakistan, as these sources were unavailable we relied on distant recall to get an idea of birth weights amongst the cases and the controls.

Association between birth weight and short body length at birth with increased rates of cardiovascular disease and non-insulin dependent diabetes later in life has been shown for the White populations; this has also been demonstrated in India.359 Although the influences that impair foetal development and lead to adult cardiovascular disease remain to be defined, there are strong pointers to the importance of foetal adaptations invoked when materno-placental nutrient supply fails to match the foetal nutrient demand.

There is no report of this association in the native Pakistani setting; the need to identify this, may particularly be relevant in the context of the strong association of CAD with insulin resistance and diabetes that has been demonstrated in this and other studies. This need is further reinforced by the knowledge that the associations between reduced foetal growth and conventional risk factors for CAD do not explain the association between reduced foetal growth and CAD, which indicates that this association must be partly mediated by processes other than the known risk factors. This is consistent with the knowledge that known risk factors cannot explain the high risk of CAD in this population. There is therefore a need to test this hypothesis further in the native Pakistani setting.

4.14 FAMILY HISTORY

4.14.1 Results

55.3% of the cases and 43.7% of the controls had a family history of one of the cardiovascular ailments, namely: hypertension, diabetes, coronary heart disease and sudden non-accidental death or a combination of these. The odds of being a case were significantly higher amongst those with a positive family history of any of the above. (MOR 1.65, 95% C.I. 1.10, 2.48; p-value=0.01).

In addition to the presence or absence of a positive family history in general, individual histories of hypertension, diabetes, CAD and sudden death were sought in the parents and siblings.

Table 4.32 Family history of coronary heart disease and its risk states

History of	Case %	Control %	MOR (95% C.I.)
Hypertension-father (p-value=0.03)			
No	65.5	70.5	1
Yes	16.0	20.0	0.90 (0.52, 1.57)
Do not know	18.5	9.5	2.06 (1.11, 3.82)
Hypertension-mother (p-value=0.04)			
No	59.0	60.5	1
Yes	25.5	31.0	0.82 (0.51, 1.34)
Do not know	15.5	8.5	2.13 (1.03, 4.41)
Hypertension-brother (p-value=0.07)			
No	67.0	75.9	1
Yes	27.0	20.9	1.59 (0.94, 2.69)
Do not know	5.9	3.2	2.64 (0.89, 7.82)
Hypertension-sister (p-value =0.11)			
No	65.7	74.0	1
Yes	27.6	22.7	1.59 (0.96, 2.65)
Do not know	6.6	3.3	2.14 (0.70, 6.54)
CAD-father (p-value=0.37)			
No	65.5	72.5	1
Yes	23.0	19.5	1.33 (0.80, 2.21)
Do not know	10.5	8.0	1.51 (0.72, 3.12)
CAD-mother (p-value=0.96)			
No	74.5	73.5	1
Yes	17.5	18.0	0.96 (0.56, 1.65)
Do not know	8.0	8.5	0.92 (0.43, 1.97)
CAD-brother (p-value =0.002)			
No	70.8	85.0	1
Yes	28.1	15.0	2.35 (1.33, 4.15)
Do not know	1.1	0.0	-
CAD-sister (p-value =0.90)			
No	89.5	88.9	1
Yes	8.8	10.0	1.02 (0.48, 2.14)
Do not know	1.7	1.1	1.50 (0.25, 9.06)
Sudden death-father (p-value =0.45)			
No	79.0	83.0	1
Yes	18.5	14.0	1.40 (0.81, 2.43)
Do not know	2.5	3.0	0.88 (0.27, 2.92)
Sudden death-mother (p-value=0.55)			
No	89.0	85.5	1
Yes	8.5	10.5	0.79 (0.41, 1.52)
Do not know	2.5	4.0	0.61 (0.20, 1.88)
Sudden death-brother (p-value=0.01)			
No	88.7	95.2	1
Yes	11.3	4.8	3.0 (1.19, 7.56)

Continued.....

History of	Case %	Control %	MOR (95% C.I.)
Sudden death-sister (p-value=0.36)			
No	95.6	97.2	1
Yes	4.4	2.8	1.75 (0.51, 5.98)
Diabetes-father (p-value = 0.63)			
No	79.0	80.5	1
Yes	10.5	11.5	0.93 (0.48, 1.80)
Do not know	10.5	8.0	1.40 (0.67, 2.95)
Diabetes-mother (p-value = 0.58)			
No	74.5	77.5	1
Yes	18.0	17.0	1.12 (0.66, 1.91)
Do not know	7.5	5.5	1.62 (0.62, 4.24)
Diabetes-brother (p-value =0.40)			
No	81.1	83.3	1
Yes	16.8	15.6	1.05 (0.58, 1.90)
Do not know	2.2	1.1	4.00 (0.45, 35.7)
Diabetes-sister (p-value =0.08)			
No	81.2	87.3	1
Yes	16.6	9.9	2.18 (1.07, 4.45)
Do not know	2.2	2.8	0.75 (0.17, 3.35)
Stroke-father (p-value =0.55)			
No	84.0	82.5	1
Yes	10.5	13.5	0.77 (0.42, 1.41)
Do not know	5.5	4.0	1.32 (0.53, 3.30)
Stroke-mother (p-value =0.82)			
No	85.0	87.0	1
Yes	12.5	10.5	1.21 (0.66, 2.22)
Do not know	2.5	2.5	1.02 (0.30, 3.53)
Stroke-brother (p-value =0.04)			
No	93.5	97.9	1
Yes	6.5	2.1	3.00 (0.97, 9.30)
Stroke-sister (p-value =0.73)			
No	97.8	97.2	1
Yes	2.2	2.8	0.8 (0.22, 2.98)
n	200	200	

The odds of being a case were found to be significantly higher amongst those with a positive family history of a brother's sudden death (MOR 3, 95% C.I. 1.19, 7.56; p-value=0.01) and highest amongst those with history of another brother suffering from CAD (MOR 2.35, 95% C.I. 1.33, 4.15; p-value=0.002)

4.14.2 Discussion

The tabulated results show a significant association of family history of sudden death and CAD in the brother with disease. It is suspected that many referred to "don't know" as "no" for history of these diseases in the parents since in that time frame facilities for screening these diseases were not widely available in Pakistan. This underestimated prevalence of these diseases in the parents.

CAD is affected both by environmental as well as genetic factors. Dominant inherited disorders such as familial combined hyperlipidaemia and heterozygous disorders such as familial hypercholesterolaemia are known to predispose to premature vascular disease. Family history is a strong risk factor for CAD and its effect is synergistic with other

cardiovascular risk factors as well.[151] Understanding the genetic basis of CAD is important as it has implications for management and prevention. Many inherited cardiovascular risk factors can be modified, such as LDL cholesterol, homocysteine and lipoprotein (a); this implies that early detection of CAD might lead to earlier intervention for genetically susceptible individuals.[152]

4.15 ANTHROPOMETRIC ANALYSIS AND BODY FAT MEASUREMENTS

4.15.1 Results

4.33: Anthropometric analysis

Anthropometric factors	Case Mean (SE)	Control Mean (SE)	MOR (95% C.I.)
Waist	90.75 (0.67)	88.47 (0.80)	1.02 (1.002, 1.04)
Waist-hip-ratio	0.94 (0.005)	0.91 (0.006)	1.06 (1.03, 1.10)
BMI	25.51 (0.26)	25.32 (0.26)	1.02 (0.96, 1.08)
Fat mass	20.26 (0.51)	19.80 (0.60)	1.04 (0.99, 1.09)
Fat percentage	28.05 (0.59)	26.24 (0.62)	1.09 (1.032, 1.14)
n	200	198	

There were no significant differences observed in the mean BMI of the cases and controls (p-value=0.53). Cases were shown to have significantly higher body fat and fat percentage compared with controls; the difference was significant for fat percentage (p-value=0.001) and marginal for fat mass (p-value=0.09). Cases however had significantly higher mean waist circumference (p-value=0.02) and waist-hip-ratio (p-value=<0.001) compared with controls. The odds of being a case significantly increased by 1.02 (95% C.I. 1.002, 1.04) with one unit increase in waist measurement.

4.15.2 Discussion

In most studies, body fat has been used to estimate overall obesity. Such indices fail to discriminate body fat from muscular and skeletal mass. In this study, analysis of BMI revealed no difference between cases and controls; body fat mass and fat percentage, on the other hand, were higher amongst cases and the difference was significant for fat percentage. This indicates that cases had higher body fat compared with controls.

However, it is the site of fat accumulation, rather than total body fat, which is considered a predominant factor for the metabolic disorders of obesity. Central adiposity, which means the preferential deposition of adipose tissue around the trunk and intra abdominally is known to be related to diabetes and CHD[138] and is a possible mechanism for high rates of both in South Asians. The only indicator of central fat accumulation measured in this study was derived from waist circumference and waist-hip-ratio; cases were shown to have significantly higher waist circumference and waist-hip-ratio compared with controls; these findings are consistent with recent reports in which South Asian expatriates have been reported to have greater amounts of total, visceral and subcutaneous fat as demonstrated by CT scan, compared with Caucasians.[344]

Body fat, particularly abdominal fat correlates with insulin sensitivity in South Asian immigrants and the presence of central obesity, has been proposed as one of the major risk factors for the development of both diabetes and cardiovascular disease.[418] Studies have shown that anthropometric measures, such as BMI, waist-hip-ratio and waist circumference, are not comparable across different racial populations.[419] This raises an important issue regarding the use of BMI across different races in the definition of obesity and predicting risk associated with obesity on the basis of BMI. There are many indications however, that in some ethnic groups, particularly of Asian origin, the risk of diabetes starts to increase rapidly at levels of BMI or waist circumference well in the acceptable range for Europeans.[420] This may imply that cut off points as recommended for European Caucasian populations (BMI >30 Kg/m^2 or waist larger than 88 cm for women and 102 cm for men) have little value in identifying Asian individuals at high risk. Our data suggests that, even at lower BMI, Pakistanis are insulin resistant and have increased total abdominal fat, which may explain their predilection for increased diabetes and CAD. Based on these observations, recently, lower cut off points for BMI and waist hip ratio have been recommended for South Asians.[421]

Increased visceral fat in Asian Indians usually has been associated with increased generalized obesity; this is often not apparent from their BMI, which is usually in the normal range as defined by standard weight tables and other readily available criteria. Recent studies have therefore identified that the simultaneous measurements and interpretation of simple variables, which include waist circumference could be used as inexpensive screening tools for the identification of men at high risk of CAD.[354]

4.16 BIOCHEMICAL ANALYSIS

4.16.1 Results

Table 4.34 tabulates mean values of biochemical risk markers in cases and controls and gives matched odds ratios.

Table 4.34 Biochemical cardiovascular risk markers in cases and controls

Biochemical parameters	Case Mean (SE)	Control Mean (SE)	MOR 95% C.I.
Cholesterol (mmol/l)	4.28 (0.07)	4.24 (0.07)	1.03 (0.86, 1.23)
LDL (mmol/l)	2.92 (0.99)	2.71(0.93)	1.20 (0.98, 1.47)
Apolipoprotein B (g/l)	0.93 (0.23)	0.91 (0.27)	1.03 (0.47, 2.35)
HDL (mmol/l)	0.73 (0.01)	0.85 (0.01)	0.09 (0.03, 0.25)
Apolipoprotein A 1(g/l)	1.09 (0.01)	1.19 (0.01)	0.06 (0.02, 0.19)
Triglycerides (mmol/l)	1.37 (0.04)	1.40 (0.05)	0.93 (0.71, 1.22)
Log Lp(a) (g/l)	-2.71 (0.07)	-2.88 (0.06)	1.24 (0.99, 1.53)
Small LDL*	3.13 (0.04)	3.02 (0.04)	1.22 (0.93, 1.59)
Glucose (mmol/l)	6.84 (0.21)	6.06 (0.11)	1.15 (1.04, 1.27)
Insulin (pmol/l)	47.42 (5.08)	26.48 (3.63)	1.01 (1.002, 1.01)
Insulin resistance*	16.78 (2.12)	8.48 (1.63)	1.01 (1.003, 1.02)
Log of CRP (mg/l)	2.05 (0.08)	1.361 (0.09)	1.52 (1.28, 1.81)
Sialic acid**	82.06 (1.12)	77.60 (0.8)	1.03 (1.01, 1.04)
Fibrinogen (mmol/l)	2.33 (0.06)	2.40 (0.13)	0.96 (0.84, 1.11)
Homocysteine (µmol/l)	20.41 (0.66)	20.14(0.68)	1.01 (0.98, 1.02)
PTH (ng/l)	67.03 (3.53)	66.40 (2.31)	1.00 (0.99, 1.01)

Continued.....

Biochemical parameters	Case Mean (SE)	Control Mean (SE)	MOR 95% C.I.
Creatine (µmol/l)	103.00 (37)	93.00 (19)	1.01 (1.00, 1.02)
Bilirubin (µmol/l)	5.62 (0.26)	7.11 (0.38)	0.92 (0.88, 0.97)
Creatinine kinase (units)	88.26 (7.75)	92.14 (5.55)	0.99 (0.99, 1.00)
Gamma GT (units)	32.22 (1.78)	30.35 (2.72)	1.00 (0.99, 1.008)
Albumin (g/l)	49.06 (0.25)	49.05 (0.26)	0.99 (0.95, 1.05)
ALT (units)	20.04 (3.3)	17.98 (1.47)	1.00 (0.99, 1.01)
AST (units)	25.38 (1.06)	26.98 (1.15)	0.99 (0.98, 1.01)
Protein (g/l)	85.52 (0.94)	85.75 (1.02)	0.99 (0.98, 1.01)
N	200	200	

* Calculated values

** Reference levels not known

4.16.2 Discussion

4.16.2.i Lipoproteins: mean total cholesterol levels were not significantly higher amongst cases (4.28, SE=0.07) compared with controls (4.24, SE=0.07), (p-value=0.74); similarly, no significant differences were observed in the mean levels of LDL between cases (2.92, SE=0.99) and controls (2.71, SE=0.93), (p-value=0.07). These levels were significantly lower than those reported for a study in a similar ethnic group in India.[406] Indeed, cases had levels of total cholesterol and LDL below currently acceptable thresholds for treatment in the UK. These results are consistent with the finding that mean serum total cholesterol levels among South Asians expatriates[310,311] in general and Pakistani expatriates,[312] in particular, are known to be lower than that of Whites in England.

These results imply greater atherogenicity of lipids at levels of LDL and total cholesterol, which are considered to be normal in the White population and may be attributed to a greater role of other lipoproteins in mediating the lipid related coronary risk in this population. With regard to LDL and total cholesterol, it may therefore be more practical to recommend the amount by which LDL should be lowered rather than specifying the absolute level of LDL cholesterol which should be achieved. However, there is very little data about the benefit, in terms of risk reduction, of lowering what would otherwise be considered "normal" levels of cholesterol in high risk South Asians compared to the risk reduction obtained from lowering abnormally high levels of cholesterol in South Asians and other populations.

In conditions where LDL levels are normal; athrogenicity of the lipid profile can also be explained on the basis of hyperapobetalipoproteinaemia (hyperapoB); in this condition, LDL levels are normal whereas LDL apoB levels are increased; LDL particles are cholesterol depleted and are relatively protein rich; a possible explanation of the decreased cholesterol to protein ratio in hyperapoB might be a shift in the spectrum of LDL towards smaller denser LDL which, is known to highly atherogenic.

Cases were found to have higher levels of LDL apoB (p-value=0.14) and apolipoprotein B (p-value=0.03) compared with controls; the findings were marginally significant. These findings are consistent with other similar findings in expatriate South Asians who are known to have higher levels of small, dense LDL compared with the White population[316,317] and with findings

both in White and South Asian populations which have demonstrated a significant association of smaller LDL with the risk of CAD. [62]

Controls in this study were seen to have significantly higher HDL (p-value=<0.001) and apo A1 (p-value=<0.001) levels compared with cases. Low HDL is a strong independent predictor of CAD66,67 and is known to be associated with insulin resistance, diabetes and abnormalities of lipoprotein metabolism, which are atherogenic. On the other hand, however, mean triglyceride levels amongst cases were not found to be significantly higher than in the controls (p-value=0.60). This is consistent with the finding that mild to moderate elevations in plasma triglyceride levels are commonly found in patients with CAD. Mild hypertriglyceridaemia, particularly when combined with low HDL is at least as powerful a predictor of CAD as isolated high LDL.75,76 This combination is often seen in the metabolic syndrome with visceral obesity, hypertension and insulin resistance as was the case in this study.

4.16.2.ii Lipoprotein (a): mean Lp(a) levels in cases were marginally higher compared with the controls (p-value=0.05). These results are consistent with other findings in the UK,[321] North America, [319,320] and Far East,[324] which show that South Asians are known to have higher levels of Lp(a) compared with other populations. Lp(a) is generally thought to be a risk factor for CAD when cholesterol concentrations are as high as in the British European populations; its atherogenicity is reduced in association with reductions in LDL cholesterol levels. In this study, lower levels of LDL indicates that the atherogenicity of Lp(a) may not have been completely manifested. On the other hand, however, CAD risk in patients with high Lp(a) is much greater in patients with low HDL than with high LDL levels.[93] HDL levels in this study have been documented to be significantly lower in cases compared with controls. Lp(a) has also been shown to be an independent predictor of future atherosclerotic events in diabetic patients in other ethnic populations; this has important implications in the South Asian context where diabetes is known to underlie the increased risk of CAD.[325,326]

4.16.2.iii Glucose, insulin and insulin resistance: glucose intolerance and hyperinsulinaemia account statistically for most of the excess prevalence of CAD in South Asians compared with Europeans; this is consistent with the hypothesis that metabolic disturbances associated with insulin resistance underlie the high coronary risk in South Asians.[18] Our findings in indigenous Pakistanis have been consistent with findings in expatriates. Mean glucose levels in this study were significantly higher amongst cases compared with controls (p-value=0.003); cases were also seen to have significantly higher mean insulin levels compared with controls (p-value=0.001). Similarly the odds of being a case were also higher amongst those with increased levels of insulin resistance (p-value=0.001).

Elevated plasma insulin is known to be an independent predictor of CAD.[349] South Asians have been reported to have higher levels of postprandial insulin compared with Europeans.[351] Insulin resistance is also a marker for many cardiovascular diseases; however the first step in the assessment of the influence of insulin resistance on the aetiology and prognosis of disease requires a simple estimate of insulin resistance. Fasting glucose/insulin has been suggested by some[422] however this conceals subjects in which insulin and glucose both rise. In this study insulin resistance was estimated by the HOMA-R parameter of homeostasis

model assessment, its expression as the product of fasting glucose and insulin yields a tangible concept of insulin resistance syndrome as it applies to patients.

Insulin resistance involves impairment of the ability of insulin to stimulate glucose uptake; defects of insulin action manifest as abdominal obesity and fasting hyperinsulinaemia. These defects have been suggested to be early metabolic markers that predict the risk of premature MI in South Asians.[345] Based on this data, it is reasonable to assume that insulin resistance underlies CAD risk in Pakistanis either directly or by its other indirect effects. It is however, unlikely, that this effect is mediated through blood pressure or HDL alone;[296] insulin resistance and the development of cardiovascular disease are probably linked through changes in lipoprotein and lipid metabolism. The characteristic lipid abnormalities of this syndrome are low HDL levels, relatively lower LDL levels, elevated triglycerides and the presence of smaller dense LDL particles. In this study, HDL levels were found to be a strong predictor of cardiovascular risk whereas small LDL was seen to correlate with insulin resistance (Pearson correlation coefficient -0.09, p-value=0.05). Support for the lipoprotein mediated coronary risk also comes from the study of the more favourable lipoprotein profile in Afro-Caribbeans compared with South Asian expatriates, who have similar prevalence rates of diabetes but lower rates of CAD.[317]

Another mechanism through which insulin resistance may mediate coronary risk may be related to inflammatory mechanisms. In a recent study, it was shown that CRP levels were higher in South Asians in comparison to the White population and that the processes underlying elevated CRP and or elevated CRP production itself were associated with about 14% increase in CAD risk in South Asians; furthermore, this was accounted for by greater central obesity and insulin resistance.[352] In this study, CRP was seen to correlate strongly with waist-hip-ratio, which is one of the hallmark abnormalities of the insulin resistance syndrome (Pearson correlation coefficient 0.12, p-value=0.01). Elevated CRP may reflect the extent of inflammation in atheromatous lesions or might contribute to atherothrombosis itself. Evidence for the latter comes from the observation that CRP has been shown to selectively bind to modified LDL within inflammatory plaques; statins have also been shown to decrease both CRP and LDL.[233,234]

The insulin resistance syndrome is seen to have both genetic and environmental causes; it has been postulated that it is more pronounced in South Asians because of low physical activity and high energy intake in a population adapted to survival under conditions of unreliable food supply and physically demanding work.[296] Defining the mechanisms that underlie insulin resistance might help to develop new strategies to treat or prevent insulin resistance and its complications. Reducing the risk of CAD and diabetes in South Asian communities is therefore likely to depend on identifying factors that influence insulin resistance and are amenable to intervention. The beneficial role of physical activity, reduction in obesity and carbohydrate intake is crucial to this concept.[423]

4.16.2.iv C reactive protein: mean C-reactive protein levels (log) of cases were significantly higher than that of the controls (p-value=<0.001). CRP is known to be an independent risk factor for CAD and has been shown to be higher in healthy expatriate Asian Indians than in European Whites; greater central obesity and insulin resistance have accounted this for;[352] adiposity is also known to be a major determinant of CRP.[379] In this study, CRP levels

correlated with waist-hip-ratio (Pearson correlation coefficient 0.12, p-value=0.01) lending further support to this hypothesis. Experimental studies suggest that abdominal adipose tissue is a major source of cytokines, including IL-6, which is an important determinant of hepatic CRP synthesis; thus central obesity identified in Pakistanis may contribute to increased CAD risk through inflammatory mechanisms.

4.16.2.v Sialic acid: mean sialic acid was significantly higher amongst cases compared with controls (p-value=0.001). Sialic acid has been shown to be a strong predictor of cardiovascular mortality.[424] It has been suggested that elevated total serum sialic acid may reflect an acute phase response involved in atherosclerosis or possibly could be related to levels of lipoprotein (a)[425] or triglycerides.[426] However, no correlation was seen between sialic acid and Lp(a), (Pearson correlation coefficient 0.08, p-value=0.09) and sialic acid and triglycerides (Pearson correlation coefficient 0.05, p-value=0.25) in this study. Sialic acid and CRP are both acute phase proteins, and a correlation between the two has been demonstrated in studies carried out on White populations,[427] CRP strongly correlated with sialic acid in this study (Pearson correlation coefficient 0.30, p=0.01). The underlying mechanism for the elevation of total sialic acid may be due to a concomitant elevation of serum acute phase proteins many of which are known to be sialated.

4.16.2.vi Fibrinogen: no difference was seen in the mean fibrinogen levels of the cases and controls (p-value=0.60). Although fibrinogen is known to be independently associated with coronary risk in the White populations, evidence in South Asians has been less convincing. Our results do not support the hypothesis that fibrinogen plays a significant role in the pathogenesis of CAD in this population.

4.16.2.vii Homocysteine: mean homocysteine levels in this study were much higher than levels reported in other studies in expatriate South Asians (11.2±3.76 in Canadian Indian Asians vs. 10±3.78 in Caucasians and 9.21±3.79 in Chinese);[286] levels were also significantly higher than the reported risk level for Caucasians (13-14 μmmol).[428] However in this study, there were no differences observed between cases and controls (p-value=0.62); this is in contrast with previously reported studies on South Asian expatriates (in the UK[369,371] and North America[286]) where plasma concentrations of homocysteine were higher in South Asians compared with Whites. Though an effect of homocysteine on the initiation of coronary artery disease cannot be excluded based on the results of this study, it seems to play little role in the progression to occlusive disease.

4.16.2.viii Parathyroid hormone: the association between elevations of parathyroid hormone (PTH) and disturbance of glucose homeostasis is well known, both in cases of primary and secondary hyperparathyroidism.[429,430] In uraemic patients, parathyroid hormone is known to interfere with glucose metabolism by either causing insulin resistance or interfering with pancreatic beta-cell function. Even in healthy normotensive subjects with glucose tolerance, insulin sensitivity is inversely correlated with plasma intact parathyroid hormone levels.[431] In this study, parathyroid hormone levels were found to be higher in cases compared with controls, the difference however was not found to be significant (p-value=0.95).

4.16.2.ix Creatinine: there is little data on creatinine as a risk factor; the risk associated with renal dysfunction is almost always ascribed to proteinuria, which was not measured in this

study. However mean levels of creatinine were found to be significantly higher in cases compared with controls (p-value=0.002). These findings are consistent with earlier recent finding which have shown that renal impairment and elevated urinary albumin excretion predicts cardiovascular disease or death in patients with type 2 diabetes.[432,433] In a recent cohort study of 12,239 postmenopausal women, the relation of urinary albumin levels to cardiovascular mortality confirms a predictive role of urinary albumin for the risk of future cardiovascular mortality, independent of hypertension and diabetes, supporting the hypothesis that microalbuminuria is a reflection of vascular damage and a marker of early arterial disease.[434] The predictive value of creatinine in cardiovascular disease has important implications for the development of screening protocols in future.

4.16.2.x Bilirubin: bilirubin has antioxidant properties[435,436] and may influence the risk of cardiovascular disease through inhibition of LDL oxidation, which is an important step in atherosclerosis. Recent studies have demonstrated that higher concentrations of total bilirubin are associated with lower risk of cardiovascular disease in men, but the pattern has been unclear in women.[437] In this study, bilirubin levels were found to be significantly higher amongst controls compared with cases (p-value=0.002). Further studies are needed to confirm these findings and to explore underlying biological mechanisms that could explain the gender differences that have been demonstrated in other studies. If confirmed by future studies, bilirubin concentration, in conjunction with traditional risk factors, could help identify those at high or low risk for CAD.

Bilirubin, sialic acid and parathyroid hormone were subsidiary analyses requested by the laboratory collaborators and were not candidates of a *priori* hypothesis. Several other analyses were also performed as part of this study. Comparison of mean values between cases and controls have been tabulated in Table 4.34; these have been tabulated for descriptive purposes and will not be discussed further in detail as there were no differences demonstrated between cases and controls for these biochemical variables. (albumin [p-value=0.98], ALT [p-value=0.58], AST [p-value=0.27], protein [p-value=0.84], creatine kinase [p-value=0.64], gamma GT [p-value=0.58]); furthermore, they also do not have a significant bearing on cardiovascular risk.

4.17 GENSINI SCORES

4.17.1 Results

The severity of CAD was also analysed by the Gensini scoring system;[390] angiograms were quantified for the extent and severity of CAD by observers blinded to other clinical details. Gensini scores were found to be significantly different in individuals with single, double and triple vessel disease and amongst those with normal coronary anatomies, (p-value=<0.001; Kruskal Wallis).

Table 4.35: Gensini scores in different categories of vascular anatomies

Vessel anatomy	Mean (SE)	95% C.I. for mean Gensini scores
Single vessel disease	36.32 (4.27)	(27.66, 44.99)
Double vessel disease	47.23 (3.61)	(40.00, 54.46)
Triple vessel disease	91.10 (4.85)	(81.48, 100.71)
Normal	0.05 (0.027)	(0.000505, 0.11)

Gensini scores were lowest for those with normal coronary angiograms and rose progressively with increasing severity of CAD.

Linear regression analysis was used to examine the relation between all risk factors measured on the continuous scale and Gensini scores by using baseline data or log transformed data depending on whether individual analytes showed a Gaussian distribution. Analysis showed that the risk of occlusive CAD also correlated with age, the duration of diabetes, waist hip ratio, low HDL, lipoprotein (a) and creatinine. Results are tabulated in Table 4.36.

Table 4.36: Linear regression analysis of Gensini scores and risk factors

Variable	Beta coefficient	SE (B)	t-value	P
Age	0.69	0.29	2.38	0.01
Diabetes-years	1.25	0.62	2.01	0.04
Waist-hip-ratio	65.87	34.90	1.88	0.06
Fat mass	-0.60	0.36	-1.63	0.10
HDL	-45.59	11.58	-3.93	<0.001
Creatinine	0.33	0.08	3.72	<0.001
Lp(a) (log)	9.46	2.87	3.29	0.001
CRP (log)	5.61	2.07	2.71	0.007

4.17.2 Discussion

Marked differences in Gensini scores were observed between those with normal coronary anatomy and those with diseased vessels indicating that all patients were assigned correctly between the groups. The Gensini method assigns a severity score depending on the geometrically increasing severity of lesions, the cumulative effects of multiple obstructions and the significance of their geographic locations. The functional concept forming the basis of this system is the hypothesis that the severity of CAD must be regarded as a consequence of the functional significance of the vascular narrowing and the extent of the area perfused by the involved vessel or vessels; the presence of an effective collateral situation may, on the other hand, modify functional significance of a severe obstruction or occlusion. Thus this system provides an accurate stratification of patients according to the functional significance of their disease. Results of univariate regression analysis of Gensini scores with several risk variables have been consistent with earlier associations of risk factors with disease when differences were sought between cases and controls. Results revealed a significant association of Gensini scores with age (p-value=0.01), duration of diabetes (p-value=0.04), HDL (p-value=<0.001), creatinine (p-value=<0.001), Lp(a) (p-value=0.001), waist-hip-ratio (p-value=0.06) and CRP (p-value=0.007). These results have been discussed along with the result of the case control analysis done by multivariate conditional logistic regression analysis in the summary of results.

4.18 MULTIVARIATE CONDITIONAL LOGISTIC REGRESSION ANALYSIS

4.18.1 Results

The following variables were tested for association with CAD in multivariate analysis: waist, waist-hip-ratio, plasma levels of HDL, apo A1, insulin, glucose, creatinine, C reactive protein, family history, fat mass, percentage of fat, past history of diabetes and hypertension, quantity of fat intake, exercise, smoking status, duration of exposure to cigarette smoke, passive smoking exposure, lifetime cigarette exposure, passive smoking as a result of a friend and spouse smoking and status of migration. For model building, the best subset selection technique was used, starting with the two most significant variables and adding other variables accordingly. A number of models containing all possible combinations of variables that were significant, according to defined criteria, were tried; models were compared through the likelihood ratio test.

Table 4.37: Results of multivariate regression analysis

Factors	Adjusted MOR (95% C.I.)
C reactive protein (log)	1.45 (1.19, 1.75) p-value = < 0.001
HDL	0.11 (0.04, 0.34) p-value = < 0.001
Waist hip ratio	1.04 (1.01, 1.08) p-value = 0.01
Family history	
No	1
Yes	1.79 (1.09, 2.93) p-value = 0.02

Cases were found to have significantly higher odds of a positive family history (p-value=0.02) adjusting for other factors in the final model. Similarly HDL (p-value= < 0.001) was found to have a significant association with disease. The odds of one unit increase in C reactive protein level (log) among cases was found to be 1.45 times compared with controls adjusted for other factors in the final model. Similarly, increase in waist hip ratio was found to be significantly associated with disease (p-value=0.003).

4.19 SUMMARY OF THE RESULTS

A number of studies have investigated CAD risk factors in migrant populations and have identified the higher prevalence of diabetes, low HDL, moderately raised LDL, and waist-hip-ratio as risk factors.[352] The contribution of smoking, lipoprotein (a) and fibrinogen have been variable between different studies.[320,406] However, studies conducted in Indian populations in situ are less frequent and have generally assessed CAD as a categorical variable in contrast to studies in migrant populations that have included quantification of severity of CAD by angiography or used surrogate markers such as carotid intima media thickness.[406] This study, similar to other studies, noted an increased prevalence of hypertension, diabetes and smoking in a native Pakistani population suffering from CAD but differed in finding a lesser relationship with total cholesterol and LDL than previous studies in India; however both total cholesterol and LDL levels were lower than those seen in the Chennai study cohort.[438] Indeed cases had levels of total cholesterol and LDL below currently accepted thresholds for treatment in the UK. In contrast to other studies, a quantitative angiography severity score

was also generated which showed that the risk of occlusive CAD correlated with age, waist-hip-ratio, low HDL and creatinine. Additional information was gained by measurement of Lp(a) and CRP.

In this study, family history has emerged as one of the strongest predictors of CAD. Familial concordance of disease can be linked to shared lifestyles as well as true genetic factors. The underlying mechanism through which family history can genetically be associated with CAD could either be lipid related or linked to the insulin resistance syndrome. There are several familial lipid disorders including familial hypercholesterolaemia (FH), familial combined hyperlipidaemia (FCH), familial hypoalphalipoproteinaemia (FHA) and familial dyslipidaemia which may be involved in mediating this risk; analysis of the data however, reveals that this is unlikely since neither LDL nor triglycerides, were strongly associated with coronary risk in this study; elevation of either LDL or triglycerides is one of the components of the above mentioned familial lipid disorders. On the other hand, the strong association with family history may be linked to Lp(a). Plasma levels of Lp(a) are 90% genetically determined; the heritability index for Lp(a) in twin studies, sib-pair analysis and family studies is highest for any of the known lipoprotein cardiovascular risk factors;[439] Lp(a) therefore appears to be a highly heritable trait. A strong genetic link is also known to exist for the insulin resistance syndrome; most of the hallmark abnormalities of the insulin resistance syndrome were strongly associated with CAD in this study, these include glucose, waist-hip-ratio, low HDL, low apo A 1, insulin and insulin resistance; small LDL, and apo B were also seen to play a marginal role. The results of this study also support the hypothesis that inflammation mediates the increased coronary risk associated with insulin resistance as was evidenced by a strong correlation of markers of inflammation with markers of insulin resistance in this study. The association of disease with the duration of diabetes and creatinine help to further highlight the role of this genetically inherited metabolic complex in the pathogenesis of CAD.

Environmental factors were also seen to influence coronary risk significantly and reinforced the earlier impressions about the importance of these risk factors in White populations. These include smoking and diet. Absence of expected associations with other dietary components such as fat could be attributed to the limitation of FFQs in giving reasonable estimates of habitual quantitative and qualitative nutritional intakes. Absence of other associations such as in the case of stress and exercise could be due to the doubtful value of self-reported personality factors and character traits. Validation studies are therefore required on existing instruments.

B. ANALYSIS OF THE ASSOCIATION OF RISK FACTORS WITH THE SEVERITY OF DISEASE

The major objective of the thesis was to highlight differences in exposure to risk factors between cases with disease and controls without disease. In addition, differences were also sought between groups with varying severity of disease. This was done initially through a comparison of study participants with different vascular anatomies classifying them in to those with single, double, triple vessel CAD and those with normal vascular anatomy. This classification allowed the inclusion, in each subgroup, of a mixture of patients with both a favourable and an unfavourable prognosis and provided marginal differentiation. However,

the ideal classification of patients with CAD should be on the basis of the severity of narrowing, cardiac performance, effectiveness of collateral circulation, the amount of myocardium jeopardized and other factors. Therefore analysis of the severity of occlusive disease was also carried out using Gensini scores.

4.20 VESSEL ANATOMY

4.20.1 Results

In addition to case-control analysis, groups with different vascular anatomies were also compared. Among the cases, 18.5% (37/200) had single vessel disease, 30% (60/200) had double vessel disease whereas 51.5% (103/200) had triple vessel disease.

4.20.1.i Age: significant differences were observed in the mean age of groups with different vascular anatomies (p-value=<0.001; One way ANOVA). The group with single vessel disease had the lowest mean age, followed by the group with normal coronary anatomy.

Table 4.38: Mean ages in categories with different vascular anatomies

Vascular anatomy	n (%)	Mean age (SE)	Median age	Range
Normal	200 (50.0)	48.21 (0.68)	47	23-72
Single vessel disease	37 (9.3)	46.27 (1.39)	46	23-65
Double vessel disease	60 (15.0)	52.80 (1.28)	53	29-73
Triple vessel disease	103 (25.8)	52.16 (0.89)	51	33-73

4.20.1.ii Years of schooling: across all groups, (single, double and triple vessel) mean years of schooling were around 10 years. No differences were observed in the mean years of schooling of those with single vessel disease (10.3 SE=0.95), double vessel disease (10.55 SE=0.56), triple vessel disease (10.35 SE=0.52) and controls (10.98 SE=0.33), (p-value=0.68; One way ANOVA).

4.20.1.iii Income: analysis of income in different groups showed no significant differences (p-value=0.25; One way ANOVA). Mean income of patients with single vessel disease was higher (£243.5; SE=84.0) than those with double vessel (£196.2; SE=49.86) and those with triple vessel disease (£140.4; SE=15.8). Mean income of the control group with normal coronary morphology was (£208.2; SE=20.7).

4.20.1.iv Waist-hip-ratio: mean waist-hip-ratio of individuals with single (0.93; SE=0.009), double (0.94; SE=0.008) and triple vessel disease (0.94 SE=0.0064) was significantly higher than that of those with normal coronary anatomy (0.90; SE=0.006), (p-value=<0.001; One way ANOVA).

4.20.1.v BMI: there were no significant differences observed in mean BMI between the groups (p-value=0.40; One way ANOVA). Mean BMI of the cases with single vessel disease was 25.83 (SE=0.66), with double vessel disease was 26.02 (SE=0.49), with triple vessel disease was 25.10 (SE=0.34) whereas mean BMI of the controls was 25.32 (SE=0.26).

4.20.1.vi Cigarette smoking: there were significant differences observed in the mean number of pack years for which the controls and cases with different vascular anatomies smoked. Controls smoked for significantly lesser mean number of pack years (122.8 SE=16.39) compared with those with single (174.4; SE=51.76) double (223.58; SE=44.29) and triple vessel disease (239.65; SE=31.52), (p-value=0.002; Kruskal Wallis).

4.20.1.vii Biochemical parameters: Table 4.39 compares biochemical risk markers of study participants with different vascular anatomies; apo A1 (p-value=<0.001, Kruskal Wallis test) and HDL (p-value=<0.001, One Way ANOVA), were seen to be significantly higher amongst those with normal coronary anatomy. On the other hand, CRP (p-value=<0.001, One Way ANOVA), fibrinogen (p-value=0.03, Kruskal Wallis), glucose (p-value=0.005, Kruskal Wallis test) and insulin (p-value=<0.001, Kruskal Wallis) rose progressively with the severity of disease.

Table 4.39: Biochemical risk markers in categories with different vascular anatomies

Biochemical values	Normal Mean (SE)	Single Mean (SE)	Double Mean (SE)	Triple Mean (SE)
Triglyceride	1.40 (0.05)	1.58 (0.13)	1.33 (0.08)	1.32 (0.05)
Apo A1	1.19 (0.01)	1.08 (0.02)	1.09 (0.02)	1.09 (0.01)
HDL	0.86 (0.01)	0.73 (0.02)	0.75 (0.02)	0.72 (0.02)
LDL	2.73 (0.06)	2.86 (0.14)	2.86 (0.12)	2.95 (0.10)
CRP (log)	1.36 (0.08)	1.96 (0.18)	2.05 (0.16)	2.08 (0.13)
Fibrinogen	2.40 (0.13)	2.25 (0.12)	2.28 (0.12)	2.39 (0.11)
Glucose	6.06 (0.11)	6.28 (0.42)	6.73 (0.34)	7.10 (0.30)
Insulin	26.49 (3.63)	48.5 (9.99)	37.07 (7.42)	53.1 (8.12)
Lp(a) (log)	-2.88 (0.06)	-2.79 (0.14)	-2.85 (0.12)	-2.60 (0.09)

4.20.2 Discussion

Mean ages of the study participants with different vascular anatomies varied significantly; those with normal vascular anatomy had the lowest mean age, followed by those with single vessel disease; the highest mean age observed, was for those, with double vessel disease and can be explained by the time taken for coronary atheromatous lesions to develop. A significant association was also found with waist-hip-ratio; those with normal coronary anatomy had a significantly lower waist-hip-ratio compared with the cases with abnormal vascular anatomy. In the comparison of biochemical values between the different groups, those with normal coronary anatomy had significantly lower glucose and CRP levels compared with those with abnormal vascular anatomies. On the other hand, values of apo A1 and HDL were much higher amongst those with normal coronary anatomy.

C. DRUG AND SYSTEMIC HISTORY

4.21 DRUG HISTORY

4.21.1 Results

Frequencies with which different cardiovascular drugs were used by the cases and controls are summarized in Table 4.40.

Table 4.40: Use of drugs

	Cases		Controls	
Drugs	%	Mean use in years (SE)	%	Mean use in years (SE)
Ace-inhibitor	28.6	1.74 (0.92)	16.0	1.04 (0.30)
Beta blocker	56.5	0.74 (0.18)	35.5	1.23 (0.31)
Aspirin	87.5	1.21 (0.17)	58.5	1.20 (0.20)
Nitrate	81.5	0.86 (0.12)	33.5	0.90 (0.24)
CCB	49.5	0.83 (0.14)	25.5	1.15 (0.21)
Anxiolytics	16.7	1.05 (0.65)	17.6	0.92 (0.25)
Sub-lingual Nitrate	38.5	1.04 (0.25)	20.0	0.95 (0.26)

Table 4.41: Use of drugs in the presence of associated conditions

Associated conditions	Cardiovascular drug	Cases %	Controls %
Hypertensives	On ACE inhibitors	30.5	32.0
	On beta blockers	56.3	47.4
	On calcium channel blockers	57.3	46.2
Diabetics	On ACE inhibitors	36.4	45.5
	On beta blockers	49.1	18.2
	On calcium channel blockers	54.5	31.5
Diabetics and	On ACE inhibitors	37.1	30.0
hypertensives	On beta blockers	51.4	20.0
	On calcium channel blockers	62.9	60.0
With angina at effort	On oral nitrates	79.8	45.3
	On sublingual nitrates	43.7	38.7
	On ACE inhibitors	24.4	17.4
	On beta blockers	60.0	44.2
	On calcium channel blockers	52.9	31.6
With angina at rest	On oral nitrates	82.5	37.3
	On sublingual nitrates	31.8	22.5
	On ACE inhibitors	24.4	17.4
	On beta blockers	52.4	35.3
	On calcium channel blockers	58.7	33.3

4.21.2 Discussion

Prescription pattern analysis of cases and controls reveals that despite active symptoms on effort warranting a coronary angiography, only 81.5% of the cases were on oral nitrates, while only 38.5% were on sublingual nitrate therapy. Even amongst those with angina at rest, 82.5% were on oral nitrates, while only 31.8% were on sublingual nitrate therapy. A similar

trend was observed for beta-blocker therapy, with 60% of those with angina at effort and only 52.4% of those with angina at rest on beta blockers. The use of calcium channel blockers amongst cases with angina at effort and at rest was 52.9% and 58.7% respectively. This pattern of drug usage indicates under utilization of nitrates, in particular sublingual nitrates in patients with evident ongoing ischemia significant enough to warrant a coronary angiogram. A similar trend was also observed for beta-blocker therapy. On the other hand, calcium channel blockers have been used in more than half of the cases with angina at rest and effort. Calcium channel blockers have also been used more frequently than beta-blocker therapy in cases with angina at rest, whereas there is clear evidence that the former is the preferred option in the absence of contraindications. Similarly, despite the undisputed role of aspirin in mortality and morbidity reduction in the setting of CAD, only 87.5% of the cases were using aspirin. Clearly, 13% of those, with documented evidence of CAD were not on aspirin; this is higher than what can be accounted for, by intolerance or hypersensitivity to aspirin. 33% and 20% of the controls were on oral nitrate and sublingual nitrate therapy respectively; this may be explainable as the majority of the controls were interviewed the day following their coronary angiogram, at a time while their nitrates were still not withdrawn.

Table 4.41 summarizes details of cases and controls with various associated conditions on different cardiovascular drugs. The results do not look at possible contraindications that may have rendered the use of a specific drug difficult, nor does this analysis take into consideration, the possibility of the presence of another disease state, which makes the choice of a particular drug useful. The table looks at the use of drugs in relation to specific individual conditions that the cases and the controls suffered from, to give an indication of the use of a relevant drug in a specific circumstance in an attempt to access the prescribing habits of the attending physicians. Results show that, of the diabetic hypertensives, only 37% of the cases and 30% of the controls were using ACE inhibitors, whereas the majority of the diabetic hypertensive cases (62%) and controls (60%) were on calcium channel blocker therapy, despite the proven advantage of ACE inhibitors in this setting. In general, prescription pattern analysis in patients with documented CAD reveals a consistent pattern of sub-optimal coverage across all major drug groups. These findings refute the lay professional opinion that patients in Pakistan are over-treated and call for aggressive appropriately targeted programs aimed at stepping up the knowledge level of physicians with regard to practices that will be useful in addressing secondary prevention.

4.22 SYSTEMIC INQUIRY

4.22.1 Results

4.22.1.i Inquiry about other cardiovascular symptoms: differences in the frequencies with which cases and controls reported having suffered from various symptoms related to cardiovascular illness are given in Table 4.42.

Table 4.42: Self-reported symptoms related to cardiovascular illness

Self reported symptoms	Case %	Control %	MOR (95% C.I.)
Palpitation	45.0	61.0	0.49 (0.32-0.76)
Dyspnoea on exertion	61.8	60.3	1.07 (0.70-1.64)
Paroxysmal nocturnal dyspnoea	19.0	34.5	0.43 (0.26-0.69)
Syncope	7.0	13.5	0.48 (0.24-0.96)
Tingling feet	17.5	16.0	1.11 (0.66-1.87)
Nocturia	27.5	19.5	1.59 (0.98-2.58)
Chest pain at rest	31.5	51.0	0.44 (0.29-0.67)
Chest pain on exertion	59.5	47.5	1.73 (1.12-2.65)
TIA	3.0	7.6	0.36 (0.13-0.99)
n	200	200	

A significant difference was observed in the frequency with which the cases experienced chest pain on exertion compared with controls (p-value=0.01); clearly this is explainable. Controls on the other hand, experienced chest pain at rest (p-value=0.002), syncope (p-value=0.03), and transient ischemic attacks (p-value=0.05) with a higher frequency; this can be explained by the more frequent reporting of functional complaints by the controls compared with cases and explains why they were referred for a coronary angiogram.

4.22.1.ii Menopause: there were a total of 64 females amongst the study participants with an equal number of cases and controls; of the 32 controls, 53.1% (17) were postmenopausal, 12.5% (4) had undergone a surgical menopause and 34.4% (11) were pre-menopausal. Similarly, of the 32 cases, 68.8% (22) were postmenopausal whereas 6.3% (2) had had a surgical menopause and 25% (8) were pre-menopausal. The odds of being a case were not significantly higher amongst those who had had a menopause (MOR 1.25; 95% C.I. 0.34, 4.66; p-value=0.73) compared with controls. A total of five females in the study were on hormone replacement therapy (HRT); in all cases, HRT was indicated for post-menopausal symptoms.

The small size of the female sample in this study and the smaller size still, of those on HRT make it impossible to make a meaningful inference from this data with regard to the effect of HRT on the risk of having CAD. The HERS data,[256] discourages the use of HRT in the secondary prevention setting and casts doubt on its use in primary prevention; in view of this evidence and the cost and logistic implications of HRT use in Pakistan, there is currently, a very limited role for HRT amongst Pakistani females and therefore, absence of relevant data in this area does not have any practical implications.

4.22.1.iii Self reported cholesterol levels: more controls compared with cases had previously had their cholesterol levels checked. The difference was found to be marginally significant (p-value=0.24).

Table 4.43: Self reported cholesterol levels

Self reported cholesterol levels	Case %	Control %	MOR (95% C.I.)
Ever checked	69.5	72.9	1
Never checked	21.0	15.1	1.50 (0.87, 2.60)
Do not know	9.5	12.1	0.86 (0.45, 1.64)
n	200	200	

Amongst those who had had their cholesterol levels checked, cases and controls self reported lipid values categorizing them into normal and high.

Table 4.44: Self reported lipid values

Self reported lipid values	Case %	Control %	MOR (95% C.I.)
Total cholesterol			
Normal	68.1	69.7	1
High	26.1	23.2	1.36 (0.68, 2.72)
Do not know	5.8	7.0	0.55 (0.13, 2.25)
Triglycerides			
Normal	79.0	78.0	1
High	15.2	14.9	1.05 (0.51, 2.14)
Do not know	5.8	7.1	0.51 (0.12, 2.11)
LDL			
Normal	91.3	91.2	1
High	2.9	0.7	26979.60($3.66x10^{5}$, $1.99x10^{103}$)
Do not know	5.8	7.2	0.33 (0.07, 1.65)
HDL			
Normal	94.2	91.4	1
High	0.0	1.4	-
Do not know	5.8	7.2	0.33 (0.07, 1.65)
n	200	200	

No significant differences ware observed between cases and controls for self reported total cholesterol (p-value=0.41), triglyceride (p-value=0.60), LDL (p-value=0.13) and HDL (p-value=0.15) levels.

More than 25% of the cases and controls were either unaware of their levels of cholesterol or did not have them checked despite having been referred for a coronary angiogram. This reflects a serious management gap.

Amongst the controls, 78.5% had their cholesterol level checked in the last one year, 13.9% had it checked a year ago whereas 7.6% had it checked more than 5 years ago. The corresponding figures for the cases were 86.2%, 10.9% and 2.9% respectively.

D. GENDER DIFFERENCES IN RISK FACTORS

4.23.1 Results

Table 4.45: Risk factor prevalence by gender (ordinal variables)

Factors	Male %	Female %
Smoking status (p-value=<0.001)		
Current smoker	19.7	3.1
Past smoker	44.1	4.7
Never smoked	36.3	92.2
Passive smoking status (p-value = <0.001)		
Not an active or passive smoker	6.2	24.6
Not an active but a passive smoker	32.5	66.7
Ever-smoker (passive or non-passive)	61.3	8.8
Duration of passive smoking exposure (p-value=0.19)		
Less than half an hour	43.2	52.9
More than half an hour	56.8	47.1
Family history of disease (p-value=0.007)		
No	53.6	34.4
Yes	46.4	65.6
Past history of diabetes and hypertension (p-value=<0.001)		
None	52.9	25.4
Only diabetes	8.0	1.6
Only high blood pressure	30.7	39.7
Both diabetes & high blood pressure	8.4	33.3
Activities while awake (p-value=0.71)		
Sedentary	37.5	32.1
Light activity	42.7	44.6
Heavy physical activity	19.9	23.2
Exercise (p-value=0.008)		
Useful	52.4	34.4
Not useful	47.6	65.6
n	334	64

Females constituted 16% of the study participants. Analysis of data by gender revealed several differences in the risk factor profiles of men and women. A significantly higher number of males were seen to be either current or past smokers (p-value=<0.001); however, paradoxically, more females compared with males had been exposed to passive smoking (p-value=<0.001). In addition, significantly more females compared with males had a positive family history of cardiovascular disease (p-value=0.007) and a past history of diabetes and hypertension (p-value=<0.001).

Table 4.46 Risk factor prevalence by gender (continuous variables)

Factors	Male Mean (SE)	Female Mean (SE)	Mean Difference (95% C.I.)
Age	49.3 (0.53)	52.1 (1.15)	-2.87 (-5.44, -0.30)
BMI	25.0 (0.19)	27.6 (0.51)	-2.56 (-3.52, -1.60)
Waist-hip-ratio	0.93 (0.004)	0.89 (0.01)	0.03 (0.01, 0.06)
Pack years of smoking	203.2 (16.4)	13.23 (7.37)	189.94 (154.43, 225.45)
Systolic blood pressure	124.4 (1.05)	141.6 (2.91)	-17.23 (-22.5, -11.95)
Diastolic blood pressure	86.15 (0.70)	91.98 (1.44)	-5.84 (-9.16, -2.51)
n	334	64	

Table 4.47 Risk factor prevalence by gender (biochemical variables)

Biochemical variables	Females Mean (SE)	Males Mean (SE)	P-value
Apolipoprotein A 1	1.22 (3.15)	1.12 (1.03)	<0.001
Apolipoprotein B	0.86 (3.12)	0.92 (1.40)	0.07
Cholesterol	4.19 (0.14)	4.28 (5.90)	0.60
Creatinine	82.81 (5.47)	101.14 (1.40)	<0.001
CRP	14.02 (3.11)	11.33 (.78)	0.23
Fibrinogen	2.46 (0.18)	2.34 (7.97)	0.58
Glucose	7.15 (0.39)	6.31 (0.12)	0.01
HDL	0.85 (3.8)	0.78 (1.15)	0.02
Insulin	61.45 (9.73)	32.23 (3.21)	0.001
LDL	2.68 (0.11)	2.84 (5.28)	0.22
Lipoprotein (a)	9.60 (1.37)	9.52 (5.52)	0.95
Small LDL	3.11 (9.49)	3.06 (3.69)	0.60
Triglycerides	1.38 (8.42)	1.38 (3.77)	0.95
Sialic acid	86.72 (1.55)	78.50 (0.79)	<0.001

Other significant differences were also observed between males and females. Females were significantly younger (p-value=<0.001), had a higher BMI (p-value=<0.001), a lower waist-hip-ratio (p-value=<0.001) and significantly higher systolic (p-value=<0.001) and diastolic (p-value=0.001) blood pressure levels whereas males were shown to smoke for significantly higher number of pack years compared with females (p-value=0.001).

4.23.2 Discussion

16% of the study participants were females; caution must be exercised however, when viewing this as the ratio of the male-female prevalence of CAD within the Pakistani population. There are several reasons why women may be underrepresented in this sample. Firstly, it is generally believed that heart disease is a man's disease; this, in addition to the frequent atypical presentation of women with CAD is a potential cause for the disease being unrecognised in women. There are also issues that centre on access to care for females in this population, which are likely to further compound, these problems. Analysis of the data reveals that 25% of the females enrolled as cases were pre-menopausal; prior to menopause the male female ratio of CAD is 9:1, this may have contributed to the under-representation of females in the sample to some extent.

Female participants in this study were less likely to be present or past smokers compared with males. This is in keeping with Pakistani cultural and Islamic religious barriers that abhor smoking amongst females. Amongst those who were smokers, women were shown to use tobacco for a higher number of years compared with men; this finding could partly be attributable to the higher mean age of the female population in this sample. Significantly higher numbers of females compared with males were exposed to passive smoking; this could be explained by the higher frequency of males smoking in the presence of women in the household.

Females were shown to have higher systolic and diastolic blood pressure levels and higher BMI compared with males but the effect of BMI on blood pressure was paradoxically observed for men and not women; this is highlighted in Table 4.48.

In simple linear regression, a one-unit increase in BMI was seen to lead to a 1.42 unit increase in systolic blood pressure amongst study participants, while amongst females this increase was by 17.22 units. In multiple linear regression however, a one-unit increase in BMI was seen to lead to a 1.02 unit increase in systolic blood pressure adjusted for gender, while among females the increase in systolic blood pressure was 14.31 adjusting for BMI. In this final model, 13.3% of the variation in systolic blood pressure was explained by body mass index and sex (Table 4.49).

Table 4.48: Univariate linear regression analysis of BMI with systolic and diastolic blood pressure in men and women

	Systolic blood pressure	Diastolic blood pressure
Study participants (BMI)	B 1.42; 95% C.I. 0.87,1.97; p-value=<0.001	B 0.62; 95% C.I. 0.28, 0.96; p-value=<0.001
Males (BMI)	B 1.42; 95% C.I. 0.87,1.97; p-value=<0.001	B 0.62; 95% C.I. 0.28, 0.96, p-value=<0.001
Females (BMI)	B 1.572E-02; 95% C.I. 1.44,1.47; p-value=0.98	B 0.29; 95% C.I. 1.01, 0.42, p-value=0.42

Table 4.49: Results of simple and multiple linear regression of systolic blood pressure and body mass index

Factor	Simple linear regression β (SE(β))	Multiple linear regression β (SE(β))
Body mass index	1.42 (0.27)	1.02 (0.27)
Sex (male as reference)	17.22 (2.26)	14.31 (2.75)
Constant (intercept)		98.84
F-statistic		27.57
Degree of freedom (df)		(2.35)
p-value		<0.001
R^2		0.13

Note: β (SE(β)) are the estimated slope and standard error of slope respectively.

For diastolic blood pressure, in simple linear regression, a one-unit increase in body mass index was seen to lead to a 0.62 unit increase in diastolic blood pressure, while among females this increase was by 5.83 units. In multiple linear regression, a one-unit increase in body mass index was seen to lead to a 0.50 unit increase diastolic blood pressure adjusting for gender, while among females, the increase in diastolic blood pressure was by 4.38 units,

adjusting for body mass index. In this final model, body mass index and gender explained 5.2% of the variation in diastolic blood pressure.

Table 4.50: Results of simple and multiple linear regression (diastolic blood pressure and body mass index)

Factor	Simple linear regression β (SE(β))	Multiple linear regression β (SE(β))
Body Mass Index	0.62 (0.17)	0.50 (0.177)
Sex (male as reference)	5.83 (1.69)	4.38 (1.74)
Constant (intercept)		73.58
F-statistic		9.90
Degree of freedom (df)		(2.35)
p-value		<0.001
R^2		0.05

Note: β (SE(β)) are the estimated slope and standard error of slope respectively.

The small sample size of females in the study population notwithstanding, there appeared to be significant differences in several other important risk states as well. Although there were no differences observed between males and females with regard to the level of activity during the daytime, 60% of the females, as opposed to 39% males reported having less than desirable leisure time physical activity, a difference that was statistically significant. The lack of physical activity in females partly explained the higher BMI. Lack of exercise and obesity also predispose to diabetes; therefore the higher rates of diabetes compared with men are part of this trend.

The clustering of risk factors within the female study participants highlights opportunities for risk factor intervention that centre on maintaining an optimal weight integrating physical activity and dietary modification with important implications for the prevention of diabetes and CAD. Physical activity should be encouraged for women in Muslim cultures in an environment and form that respects religious proscriptions. Support from Islamic religious leaders is crucial in endorsing the need for more exercise and in encouraging full participation in Muslim cultures.

5 SUMMARY

Summary

It is not widely realized that at present, developing countries contribute a greater share to the global burden of CVD than the developed countries and nothing less than an explosion in this disease burden is projected over the coming years, termed as the impending global pandemic of cardiovascular diseases. South Asia represents more than one quarter of the developing world and according to World Bank $1 a day estimates, is one of the poorest and most deprived regions in the world. This gap is also evident by comparing the South Asian GNP per capita of US $393 to $1250 for rest of the developing world. For this vast mass of mankind, the average public sector expenditure on health (as a percentage of the GDP) was 0.85% in 1997 compared with 2.0% for rest of the developing world.

Located in South Asia, Pakistan has a population of 140 million of which 64% is rural; Pakistan's economy and health resources, on the other hand, translate into tough health priorities and therefore, the impact of the cardiovascular disease epidemic is likely to have grave implications. Clearly, the first step in addressing this issue would be to identify the risk factor profile of this population; this must include research that serves to inform policy and subsequently, the initiation of preventive programs, which could control the CVD epidemic through cost-effective strategies that are feasible and hold the promise of early impact. Health prevention and promotion are the only affordable solutions for the vast populations of South Asia; classical medical interventions are, and will continue to remain, inaccessible to the majority of the population in the short to medium term.

Insight into the South Asian coronary risk comes from the study of expatriate South Asians, which as an ethnic entity, have increased prevalence and definite evidence of excess mortality from CAD compared with any other ethnic group. This excess CAD risk cannot be explained entirely in each South Asian sub group by conventional risk factors alone. Data reveals that expatriate South Asians do however, suffer from increased risk of type 2 diabetes, together with adverse fat distribution, hyperinsulinaemia and insulin resistance relative to other ethnic groups. This, known as the insulin resistance syndrome, is known to underlie the increased coronary risk in South Asian populations. Several mechanisms have been hypothesized to link insulin resistance with coronary risk; these centre on lipid mediated and inflammatory mechanisms; however further research is needed to clarify these issues. It may be true that the overall high CAD risk in South Asians cannot be explained on the basis of conventional risk factors alone, but conventional risk factors for CAD such as smoking, high blood pressure, dyslipidaemia and diabetes are highly prevalent in some South Asian ethnic subgroups and will remain the key, if not the only elements of the risk factor model in this population. For the expatriate population, migration has also been shown to confer a higher risk of CAD through the acquisition of westernized behaviours. The likelihood of increase in insulin resistance, its expression as frank diabetes and glucose intolerance probably increases on migration. The identification of other emerging or protective risk factors is therefore important so that new approaches to the prevention of CVD may be developed because a single risk factor model and prevention strategy is not applicable to all populations.

In Pakistan, population-based risk factor prevalence data is available, which indicates high prevalence rates of conventional cardiovascular disease risk factors, with over 30% of the population, over 40 years of age affected. However, no attempt at identifying risk factors amongst those with established CAD had ever been undertaken; this gap needed to be bridged since definition of the risk factor profile of a population is crucial to set targets for intervention and goals for preventative initiatives. The ideal model would be a well-designed multi-centre prospective cohort study in the Pakistani population. However, issues of cost, time and institutional capacity make such an effort unlikely in the near future. Similarly the 10-20 year lag time for results from such studies makes an urgent case for short to medium term strategies to counter the impending CVD pandemic. It is here, that well designed case-control studies can build upon existing data. The study described within this thesis, "the study of the risk factors for coronary artery disease in a Pakistani population" is therefore a step in identifying the coronary risk factor profile of the Pakistani population.

This study was the first ever study of coronary risk factors in a Pakistani population, examining risk factors predisposing to occlusive CAD in a matched case-control study of patients presenting with chest pain to two tertiary referral sites in Pakistan. 400 patients were recruited with ethical consent who underwent coronary angiography from 1998-2000 for presenting symptoms suggestive of CAD. Two groups were selected based on the presence of significant CAD as defined by 50% stenosis in one or more coronary arteries and an age and sex matched control group, presenting with similar symptoms, but with no angiographic evidence of disease. A detailed cardiovascular risk profile was obtained through a structured interview; this included data on demographic and socio-economic status and lifestyle, personal and family history of CAD. The components of the questionnaire were compiled with the use of previously validated questions included in other studies. Several techniques of observation were also used as methods of data collection; these included triplicate measurement of blood pressure using mercury sphygmomanometer, measurement of waist-hip-ratio and total body fat estimation by the OMRON BF 300 impendence system. Fasting blood samples were obtained for determination of biochemical risk factors including baseline renal function, liver function, glucose, insulin, lipid and apolipoproteins, lipoprotein (a), homocysteine, C-reactive protein, and fibrinogen. Biochemical analytes were measured by automated methods on Cobas Mira and Fara 2 analyzers, Behring BN2 nephlometer and the Corning ACS 180 immunoassay system. Insulin resistance was calculated by the HOMA-R method 4. Angiograms were also quantified for the extent and severity of CAD by the Gensini scoring system by observers blinded to other clinical details; this method assigns a different severity score depending on the geometrically increasing severity of lesion, the cumulative effects of multiple obstructions and the significance of their geographic locations.

Statistical analysis was initially conducted between cases and controls. Univariate analysis using conditional logistic regression of each variable was carried out and matched odds ratios with 95% confidence interval were obtained. Conditional multiple logistic regression technique was used for building the final model; analysis was conducted by the best subset selection technique. In addition to case-control analysis, linear regression and correlation were also used by using baseline data or log transformed data depending on whether individual analytes showed a Gaussian distribution, to determine the relationship of Gensini scores and the exposure variables measured on the continuous scale and the nature of the relationship. The statistical significance of the relationship was computed by calculating p-

values and regression coefficients. Statistical analyses were conducted using SPSS 7 for Windows 98 and GB Stat 7.0 (Dynamic Microsystems, Silver spring, Maryland, USA).

In univariate conditional logistic regression analysis, significant associations of disease were found with several risk factors; these included current and past status of smoking, passive smoking, exposure to environmental tobacco smoke as a result of the spouse smoking, lifetime cigarette exposure, socio-economic stress, sedentary habits, history of diabetes and high blood pressure, consumption of fatty foods, family history, fat percentage, waist circumference, waist-hip-ratio, low apolipoprotein A1, low HDL, lipoprotein (a), glucose, insulin, insulin resistance, CRP, sialic acid, creatinine and bilirubin. In multiple conditional logistic regression however, significant associations were found only with low HDL, family history, C reactive protein, and waist hip ratio after adjusting for all other factors in the model. In addition, linear regression analysis of Gensini scores with risk factors showed that the risk of occlusive CAD also correlated with age, the duration of diabetes, waist-hip-ratio, low HDL, lipoprotein (a) and creatinine.

In this study, family history has emerged as one of the strongest predictors of CAD. The underlying mechanism through which family history can be associated with CAD could either be lipid related or linked to the insulin resistance syndrome. There are several familial lipid disorders including familial hypercholesterolaemia (FH), familial combined hyperlipidaemia (FCH), familial hypoalphalipoproteinaemia (FHA) and familial dyslipidaemia which may be involved in mediating this risk; analysis of data however, reveals that this is unlikely since neither LDL nor triglycerides were strongly associated with coronary risk in this study; elevation of either LDL or triglycerides is one of the components of the above mentioned familial lipid disorders. On the other hand, the strong association with family history may be linked to Lp(a). Plasma levels of Lp(a) are 90% genetically determined, therefore, it appears to be a highly heritable trait. A strong genetic link is also known to exist for the insulin resistance syndrome; most of the hallmark abnormalities of the insulin resistance syndrome were strongly associated with CAD in this study, these include glucose, waist-hip-ratio, low HDL, low apo A, insulin and insulin resistance; small LDL and apo B were also seen to play a marginal role. The results of this study also support the hypothesis that inflammation mediates the increased coronary risk associated with insulin resistance as was evidenced by a strong correlation of markers of inflammation with markers of insulin resistance. The association of disease with the duration of diabetes and creatinine help to further highlight the role of this genetically inherited metabolic complex in the pathogenesis of CAD.

Environmental factors were also seen to influence coronary risk significantly and reinforced the earlier impressions about the importance of these risk factors based on studies in White populations. These include smoking, diet and physical activity. Favourable environmental and lifestyle choices can also be seen to significantly influence coronary risk through its interaction with the insulin resistance syndrome.

This is one of few studies performed in native South Asian populations as opposed to migrants to developed countries and the first study to use quantitative angiographic data in this population as opposed to cross-sectional epidemiological cohort study methods. It differs from other studies in showing that despite a predominantly meat-eating diet, populations in Pakistan have similar total cholesterol and LDL to Hindus from North India (TC=166 mg/dl)[406]

and lower values than those reported from Southern India (240 mg/dl),[438] where there is a high prevalence of vegetarianism but also a high saturated fat intake.[440] The study also found marked differences in anthropomorphic and insulin resistance associated variables between cases and controls. Further biochemical analysis will be conducted on the stored blood samples for risk variables associated with insulin resistance and once genetic risk factors for the metabolic syndrome have been clarified, this group will prove ideal to assess the contribution made by these risk factors in a native South Asian population.

The public health significance of the data gathered is that despite levels of LDL considered adequate as targets in Europe (3 mmol/l)[441] and the USA, (2.5 mmol/l),[69] many patients in Pakistan show the presence of significant established CAD. In fact, in this population, HDL is a far stronger risk factor. This suggests that drug recommendations based on the European/American practice recommending the use of statins as first-line agents may not be entirely correct and highlights the need to redefine the currently practised therapeutic approach to CAD management in this population to fit local needs.

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