Pak **Heart J**

PREVALENCE AND ROLE OF DIFFERENT RISK FACTORS WITH EMPHASIS ON GENETICS IN DEVELOPMENT OF PATHOPHYSIOLOGY OF CORONARY ARTERY DISEASE (CAD)

Sana Ashiq[1], Kanwal Ashiq[2], Shabana[3], Saleem Ullah Shahid[4], Mehwish Qayyum[5], Haleema Sadia[6]

[1,3,4]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan
[2,5]Faculty of Pharmaceutical Sciences, Superior University, University of Punjab, Lahore, Pakistan
[6]Department of Biotechnology, BUITEMS Quetta, Pakistan

Address for Correspondence:

Kanwal Ashiq

Faculty of Pharmaceutical Sciences, Superior University, University of Punjab, Lahore, Pakistan

Emails: kanwal.ashiq@superior.edu.pk

ABSTRACT

To focus on the prevalence and role of different risk factors contributing in pathophysiology of coronary artery disease (CAD). Relevant articles published between 2006 and 2018 were studied with the help of various data bases including Google scholar, PubMed, Medline, Springer link, and Science direct. Articles published only in indexed journals were taken in account to ensure the credibility of data. Coronary artery disease (CAD) is a silently progressive chronic disorder which generally establishes overtime to an advance stage till the symptoms appear. In high income countries, the mortality rate has declined since 1980 whereas, middle and low income countries bear three quarters of the global CAD burden. South Asians are at a greater risk and the prevalence is 50% to 300% higher than the rest of the world. It is a multifactorial disorder and arises from an interaction between environmental and genetic factors. The conventional CAD risk factors (CRFs) include age, gender, blood lipids, smoking, blood pressure and diabetes. Most of the CAD risk factors are modifiable by targeting lifestyle changes, drug intervention and prior identification of those who are at high risk of developing disease. The variability in disease susceptibility in individuals exposed to similar environmental factors and having almost same CRFs can be attributed to the genetic variations. Genetic testing may improve discrimination over and above the CRFs.

Key Words: Coronary artery disease (CAD), Risk factors, genetic testing, Oxidative and inflammatory pathways, Diabetes, Hypertension, Obesity.

INTRODUCTION

The word cardio relates to heart and vascular, so cardiovascular diseases (CVDs) refer to a group of disorders related to heart and blood circulation. Among CVDs, coronary artery disease (CAD) is most common in the developing nations where it is a major threat and leading cause of mortality.[1] Coronary artery disease is also known as coronary heart disease (CHD) or Ischemic Heart Disease (IHD), in which constriction of blood vessels leads to the poor oxygen supply to the heart and subsequently results an imbalance state between oxygen demand and supply.[2] Regulation of blood flow to coronary arteries occurs through two major blood vessels (i) Resistance vessels and (ii) Epicardial conduit vessels. Resistance vessels oppose the blood flow as its diameter decreases less than 300 μm. Epicardial conduit vessels show little resistance to blood flow and driving pressure along this vessel is maintained in such a way that only a little blood pressure falls in distal epicardial vessels. This driving pressure is a key regulator of myocardial blood flow.[3] In coronary artery disease, atherosclerosis plays a key role in which obstruction of blood flow occurs due to some systemic disorder including lipid metabolism and inflammatory procedure. This can be subclinical disease in which plaque remains asymptomatic while in some cases it may be vulnerable due to thrombosis.[4] Atherosclerosis in the blood vessels leads toward the development of three major clinical presentation of disease: Stable angina which develops due to stenosis, Unstable angina that occurs due to the disruption in blood circulation by aggravation of thrombosis and Myocardial infarction which is the most serious condition that involves permanent obstruction of the blood circulation.[5] The CAD may manifest as acute (e.g., myocardial infarction) or chronic condition (e.g., stable symptoms due to ischemia).[6] The spotting of disease depends on clinical symptoms like difficult breathing, chest pain and sudden rise and fall in the blood troponin (cTns) level with variation in electrocardiography (ECG).[7] Furthermore psychological and different social factors such as work pressure, poor lifestyle, financial stress may also increase the chances of the onset of the disease.[8]

The main objective of current review is to put main emphasis on the prevalence and role of different risk factors contributing in pathophysiology of coronary artery disease (CAD). The study was carried out by reviewing the relevant articles published between 2006 and 2018 were considered with the help of various databases including Google scholar, PubMed, Medline, Springer link, and Science direct. To ensure the credibility of data the articles which wereonly publishedin indexed journalswere considered.

PREVALENCE AND MORTALITY RATE

According to the data presented by World Health Organization (WHO), the global burden of coronary artery disease in year 2002 was 7.1 million which was predicted to be raised to 11.1 million by year 2020. Worldwide, so far the most affected region where this disease is the major cause of death is the Indo-Pakistan subcontinent.[9] The likeliness of the occurrence of CAD In this region is four times high (40% higher mortality rate) as compared to the other European countries.[10] As mentioned earlier adoption of modern life style and increasing rate of metabolic disorders are the main grounds for high prevalence of disease. The metabolic disorder rate area is about 40% in the elderly population of south

Indian urban region. One of the main predisposing factors of CAD is Obesity as in 2001 Obesity National Health survey has reported that the prevalence of obesity in Pakistan is about 13% in males and 23% in females.[12] As far as mortality of Coronary artery disease is concerned, a great variation is found among Asian continents i.e. low mortality rate in east and high mortality rate in south Asian countries.[13] The report of World Health Organization (WHO) states it to be the third most highest prevailing fatal disease on the globe.[14]

The most effected country by CVS diseases worldwide according to American Heart Association and World Health Organization, where it serves to be the first cause of mortality is United States.[15] Ischemic heart disease put a serious economic burden on public health systems. Due to mortality from congenital heart diseases, diabetes and stroke, WHO reported that India has lost 8.7 billion US dollars from national income.[16] Worldwide, the annual mortality rate due to non-communicable diseases (NCD) is about 38 million whereas the low and middle income countries account approximately 70-80% of this number. World health organization has reported in country profile (2014) of non-communicable diseases (NCD), that in Pakistan the communicable diseases account for about 38% while non-communicable disease contribute about 50% of the overall disease burden. Among all NCDs, cardiovascular disease contributes almost 19%, furthermore 1 in every 4 adults are presented with coronary artery disease.[17,18]

During 1960 to 2002, the Asian countries have displayed increase rate of about six fold in coronary artery disease in the urban areas while two fold rise in rural areas.19South Asian countries (India, Pakistan and Bangladesh) have about 22% of world population (among the top ten most populated countries in world) and incidence of coronary artery disease appeared 10 year earlier as compared to the rest of the world.[20] Over past 30 years downward trend in the progression of CAD is seen in various developed countries like United States, Canada, France, Australia. The major cause behind this decrease rate was awareness among people about risk factors related to this disease.[21]

RISK FACTORS

Both environmental and genetic factors have played a key role in advancement of coronary artery disease.There are two broad categories of conventional risk factors (CRFs):

1. Modifiable risk factors

2. Non –modifiable risk factors.

The prevalence of the conventional risk factors also differed among different countries.[22]

The modifiable risk factors include following

a) Smoking

b) Obesity

c) Diabetes mellitus

d) Hypertension

e) Dyslipidemia

f) Stress and depression

g) Sedentary life style

Non-modifiable risk factors are like

a) Male gender

b) Family history of premature coronary artery disease

c) Age > 40 years

d) A third category which is known as partly modifiable includes risk factors include menopause and personality type.

e) Other non-traditional risk factors are serum Apo lipoprotein-a, interleukin-6, highly sensitized c-reactive protein (hsCRP), myeloperoxidases, homocysteine and fibrinogen levels.[23]

MODIFIABLE RISK FACTORS ROLE IN CORONARY ARTERY DISEASE

Smoking and tobacco consumption

Overall there is 70% increase in the risk of death due to cardiovascular diseases in smokers as compared to non-smokers. In 1999, occurrence of about four million deaths was seen and the main cause behind these deaths was the use of tobacco.[24]

Smoking induces coronary artery disease by triggering of the following mechanisms:

a) By starting endothelial damage and impairing its function.

b) It reduces the level of high density lipoprotein (HDL).

c) In combination it also increases the intensity of proatherogenic lipids and also causes initiation of oxidation of these lipids.

d) It causes initiation of inflammation which cause development of procoagulation in circulation.[25]

Obesity and dyslipidemia

One of the main independent factor that contributes to the cause of coronary heart disease is Obesity. It is considered as a major cause of death particularly among south Asian population.[26] Obesity is responsible for about 23% of global burden of coronary heart diseases. Moreover the high levels of low density lipoprotein (LDL) with low levels of high density lipoprotein (HDL) are considered as a major risk factor for causing cardiovascular diseases. Obesity can be defined as when body mass index (BMI) is more than 30.[27] Obesity promotes the coronary artery disease by exacerbating factors: hypertension, plasma lipids and inflammation which puts a burden on heart and effecting both heart function and structure.[28]

Diabetes mellitus

Worldwide, diabetes mellitus prevalence increased around 177 million in 2000 and will increase to approximately 360 million cases by year 2030. According to EDIC (Epidemiology of Diabetes Interventions CAD) patient who have aggressive glucose control have less chance of developing cardiovascular diseases.[29] Diabetes is defined as more than 126 mg/dl of plasma glucose level in fasting. In type 2 diabetes mellitus, insulin sensitivity impairs due to release of free fatty acids (FFAs) from adipose tissue. Free fatty acids initiate production of reactive oxygen species which cause defect in activation of Phosphatidylinositol-3-Kinase and Protein Kinase B (PI3K-Akt) signaling and insulin receptor substrate (IRS-1), which ultimately leads toward the down regulation of insulin responsive glucose transporter 4 (GLUT-4).This results defect in phosphorylation of endothelial nitric oxide synthase (eNOS) causing endothelial dysfunction and increased vascular thickness which is an important predictor of cardiovascular disease.[30]

Hypertension

Hypertension or high blood pressure is a condition in which when cardiac muscles of heart pumps the blood there is increased force of blood against the wall of blood vessel. The major risk factors which lead towards the hypertension are kidney dysfunction, obesity, high triglycerides and high sodium to low potassium levels.[31] Arterial hypertension is stated as diastolic blood pressure which exceeds more than 90 mmHg or when systolic blood pressure is more than 140 mmHg. Hypertension is considered as a leading risk factor for cardiovascular diseases as it causes the disruption of two major pathways: hyperactivation of renin-angiotensin aldosterone system (RAAS) and increase in vascular tone.[32] Two major effectors molecules are renin and aldosterone. Active form of renin is produced by proteolytic and non- proteolytic cleavage of pro-renin. Then angiotensinogen is cleaved by renin into angiotensin I. This is cleaved by angiotensin-converting enzyme into angiotensin II, Then neutral endopeptidases or angiotensin converting enzymes 2 (ACE2) also cleave this angiotensin I into angiotensin-(1-7) which opposes angiotensin II thus favors the vasodilation of cardiac tissue.[33] Hypertension causes no symptoms thus also known as silent killer. Renin-angiotensin aldosterone system is an essential regulator of hemodynamic stability as it controls electrolyte balance and circulating volume in human body. The most important therapeutic target is based on RAAS inhibitors such as AT1 receptor blockers (ARBs) and angiotensin-converting enzyme (ACE) inhibitors. ACE inhibitors causes the vasodilatation by lowering the degradation of bradykinin hence contribute towards the release of nitric oxide and prostaglandins while both inhibitors block angiotensin II.[34]

Sedentary life style

Coronary artery disease is also influenced by the adaptation of modern life style factors. Smoking is among the most notorious environmental risk factor for disease. Obese or overweight adult persons and high Body mass index (BMI) in late childhood is a major cause of disease. Moderate physical activity also proves beneficial in reducing the risk for development of disease.[35]

NON-MODIFIABLE RISK FACTORS ROLE IN CORONARY ARTERY DISEASE

Age and gender

Age is an another independent risk factor for developing coronary artery disease and advance aging accelerate the global burden of acquiring the cardiovascular diseases.[36] Reduced physical exertion and activity among older age groups is also considered as prime cause of developing heart diseases. Physical inactivity is reported by American Heart Association as a most contributing factor in the development of heart diseases.[27] The prognosis of cardiovascular diseases in female is late as compared to males i.e. about seven to ten years.[37] This gender differences is due to the variation in hormonal status as estrogen hormone in women

have protective antioxidant effect.[1] Estrogen mediates the vasodilation of endothelial blood vessels. This hormone has several regulating factors on metabolic disorders like inflammatory process, coagulant system and also plays an important role during lipid metabolism. But after menopause there is a decline in these estrogen levels and due to this hormonal fall there is a susceptibility of an increased risk of developing metabolic syndrome alongside cardiovascular diseases. This is because the disturbance in lipid metabolism occurs and central obesity with increase visceral fat also develops after menopause.[37]

Genetic basis of coronary artery disease

Atherosclerosis which is one of the key processes behind CAD is caused by both genetic and environmental factors. Atherosclerosis is characterized by inflammation of large arteries in which inflammatory, lipid molecules and fibrous element slowly start depositing in vessels walls. There is a transformation of macrophage into foam cells that happened as a result of binding of Oxidized LDL to macrophage along with release of interleukins which ultimately leads toward the development of fatty streaks. This could be characterized as atherosclerotic lesion in which there is deposition of foam cells in sub endothelial cells.Genetic factors are responsible for causing disease by two major mechanisms either by its direct intervention or by exerting their effects through cardiovascular risk factors.[38] During each stage of atherosclerosis cytokines plays a very important role and also mediate survival and proliferation of the cells which are involved in plaque formation (Table 1). Like IL1-1β is a pro-atherogenic it causes atherosclerosis due to causing oxidative stress and arterial inflammation.[39] The endothelial and smooth muscle cells apart from cytokine also proliferate due to inflammatory and biochemical modifications which ultimately progress toward the formation of plaque. The formation of plaque is then preceded by the rupturing of plaque which initiates the process of thrombosis i.e. facilitated due to the interaction between platelets, coagulation proteins and procoagulant material within the core of plaque.[5]

Table 1: Major Inflammatory Genes Involved in Pathogenesis of CAD

GENE	CYTOKINE
IL1A, IL1B, IL1RN	IL-1 system
IL-10	IL-10
IL-6	IL-6
TNF	TNF α
LTA	TNF α
TGFB1	TNF α1
TGFB2	TNF α2
SELE	E-Selection
SELP	P-Selection

There are several metabolic pathways which worksin an unorganized and improper manner during this disease and contributes in the disease advancement. The major pathways by which these genes presents their involvement in causing coronary artery disease are by regulating the lipid metabolism, oxidative stress, folate metabolism, DNA damage, Renin-angiotensin pathway and various other such inflammatory process.[40] Genes that involved in pathogenesis of CAD are presented in table 2,3,4 and 5.[5]

Table 2: Major Inflammatory Genes Involved in Pathogenesis of CAD

Metabolic pathway	Gene symbol	Gene name	Chromosomal location
Lipid metabolism	LPL	Lipoprotein Lipase	8p22
	APOE	Apolipoprotein E	19q13.2
	APOB	Apolipoprotein B	2p24 -p23
	APOA1	Apolipoprotein A -I	11q23 -q24
	APOA5	Apolipoprotein A -V	11q23
	APOC3	Apolipoprotein C -III	11q23.1 -11q23.2
	ABCA1	ATP -binding cassette A -I	9q31.1
	CETP	Cholesteryl transfer protein	16q21
	LIPC	Lipase	15q21 -q23
	LPA	Lipoprotein -a	6q26
	LDLR	Low density lipoprotein receptor	19p13.3

Table 3: Gene Involved in Endothelial Integrity

Metabolic pathway	Gene symbol	Gene name	Chromosomal location
Endothelial integrity	NOS3	Nitric oxide synthase 3	7q36

Table 4: Genes Involved in Renin Angiotensin Pathway

Metabolic pathway	Gene symbol	Gene name	Chromosomal location
Renin angiotensin pathway	ACE	Angiotensin converting enzyme	17q23.3
	AGT	Angiotensinogen	1q42-q23

Table 5:Gene Involved in Hormonal Pathway

Metabolic pathway	Gene symbol	Gene name	Chromosomal location
Hormonal	ESR1	Estrogen receptor 1	6q25.1

Other metabolic pathway involved in CAD

Gene involved in oxidation reduction pathway

Paraoxonases gene (PON) gene:

Paraoxonasesas the name indicates are group of enzymes which like most other enzymesare substrate specific. These are basically lactonases (acyl-homoserinelactonase). There are three types of paraoxonases which are PON1, PON2, and PON3. All these three enzymes metabolize compounds which are derived from arachidonic acid. Its lactonases activity enabled it to degrade and breakdown lipophilic lactones and thus it has ability to breakdown oxidized lipids in lipoproteins and cells. This gene property has enabled it to provide protection against cardiovascular diseases. The location of PON2 is intracellular while PON1 and PON3 are extracellular and associated with high density lipoproteins (HDL) which are present in many tissues as well as in circulation.[41]

PON1 gene:

The identification of this gene was first done by Abraham Mazur's in early 1950's and it was named after its ability to hydrolyzed paraoxon which is a toxic metabolite of insecticide parathion.[42] PON1 is responsible for provision of protection against atherosclerosis by preventing lipid per oxidation and this serves as one of its key role. It is a calcium dependent glycoprotein which is basically synthesized in liver and then secreted into plasma where it is associated with high density lipoproteins (HDL).[43] These enzymes are important in elimination of carcinogenic lipid soluble radicals by inhibiting the oxidation of low density lipoproteins and high density lipoproteins. Thus it blocks the formation of oxidized LDL and LDL derived oxidized phospholipids and also prevents oxidation of phospholipids of HDL.[44] PON1 have two major activities which may differ from each other in their mechanisms: one is the esterolytic and other is hydroxyperoxide activity. Its hydrolytic activity facilitates the degradation of esterified lipid. Basically the paraoxonases 1 avert the two key major pathways in atherosclerosis one is plaque formation and other one is foam cell formation. MCP-1 and M-CSF, the two major chemo attractant enables the process of foam cell formation when MCP-1 and M-CSF helps in the passage of monocytes through artery wall where they get converted into macrophage. LDL gets oxidized by reactive oxygen species (ROS) as they enter the blood vessels. Then macrophage has

scavenger receptors for this oxidized low density lipoprotein which leads to its transformation into the foam cells. PON 1 resists all these process by the following routes:

1. PON1 decreases the level of oxidized low density lipoproteins by inhibiting LDL oxygenation by ROS.

2. It also inhibits foam cell formation by stopping the release of chemo attractant.

3. It facilitates and helps in increase efflux of cholesterol from macrophages.[45]

PON1 structure:

The enzyme commission of the International Union of Biochemistry and Molecular Biology classified as an aryldialkylphosphatase. It is a glycoprotein which has 43KDa molecular mass and composed of 354 amino acids. Along its association with HDL it is also related to apo A1 and clusterin. X-ray crystallography revealed its structure which is a six bladed propeller which has a lid covering the active site passage along with it contains 2 calcium ions. One connected calcium ion is used for its stabilization of the whole structure while other is important for its activity.[46]

PON1 gene structure:

PON 1 is located on q arm of chromosome at position 21.3-22.1(49) where as PON2 and PON3 are also located on the same arm of chromosome adjacent to PON.[43] It is 26KB in size. There are total nine exons which are located in coding regions along with splice donor and accepter site. Polyadenylation signal sequence is absent in this gene. It has eight introns (non-coding) region while no canonical TATA box is present at 5 UTR. Sterol regulatory binding protein 2 (SREBP2) and specificity protein 1 (Sp-1) has binding sites on the promoter which up-regulate the level of PON1 in the presence of statins.[46]

Single nucleotide polymorphisms (SNPs) in PON1 gene

A total of two hundred single nucleotide polymorphisms have been reported in this PON1 gene.[47] (Table 6)

Single nucleotide polymorphism (Q192R) in coding region of PON1 gene

Table 6: SNP's Present in Different Regions of PON1 Gene.

Gene region	SNPs reported
Exonic region	5 SNPs
Intronic region	171 SNPs
Promoter region	7 SNPs
3´-untranslated regions	15 SNPs

Polymorphisms both in coding and promoter region of gene paraoxonases1 are functional SNPs.[48] PON1 gene coding region has two major polymorphisms in its coding region, one happens to be L55M where at position 55 methionine replacement occur instead of leucine.[49] The other polymorphism is present on exon 6 which is a Q192R where a glutamine (Q) to arginine (R) substitution occurs at position 192 or A G nucleotide substitution of Q192 (CAA) or R192 (CGA).[50] The catalytic activity of gene paraoxonases 1 is effected by single nucleotide polymorphism.[51] This polymorphism effect on activity is substrate dependent.[46] The glutamine variant has higher hydrolytic activity of substrate paraoxon as compare to arginine variant.[52] The R192 isoform is less stable in preventing oxidation of low density lipoprotein as compare to Q192. Thus R allele carriers are more vulnerable to cardiovascular diseases.[51] This gene polymorphism is also a risk factor and presents a major concern for developing other diseases like type 2 diabetes and inflammatory bowel disease, Parkinson's disease.[53] Though there is a difference among different ethnic group populations as north American Caucasians revealed R-allele as a risk allele for CAD while opposite results are obtained from Spanish, Korean and British Caucasians.[43]

Inflammatory pathways

Atherosclerosis is characterized by the chronic inflammation of coronary artery wall. The primary reason is accumulation of oxidized lipid in inner walls of arteries.[54] Inflammatory processes currently serves as a key cause for developing atherosclerotic plaque. A large number of inflammatory cells and proinflammatory cytokines (IL-1, IL-6and TNF) are present in the plaque that regulate inflammatory processes along with it involvement in cardiovascular diseases by changing the plaque stability.[55] Inflammatory cells which are important for the progression of atherosclerotic plaque produce cytokines like interleukin-1; interferon gamma and tumor necrosis factor.Various interleukins are related to arterial wall inflammatory process including Interlekin-6 which manages the expression of adhesion molecules and endothelial cell proliferation on arterial wall.[54]

Interleukin-1 gene cluster

Immune processes are regulated by cytokines which are short and small acting. The inflammatory process though is most importantly regulated by the interleukin-1 (IL-1), tissue repair and cell growth. IL-1 has three major members in this group: Interleukin-1 A (IL-1A), Interleukin- 1 B (IL-1B) and IL-1 RN (IL1RN). These three genes are present on q-arm of chromosome two (2q13-21) located within a 430 kb region. IL-1A and IL-1B encode the two agonists IL-1α and IL-1β respectively while IL-1 RN encodes one antagonist (IL-1Ra).[56] Both interleukin-1A and B bind to IL-1 receptor and are proinflammatory cytokines while an inhibitor of binding of this interleukin to receptors are IL-1 receptor antagonist. IL-1RN gene has 86bp variable numbers of tandem repeats (VNTR) which causes length variation within intron 2. IL-1 receptor antagonist expression depends upon the IL-1RN gene.[57] This interleukin 1 family has various roles in coronary artery disease development by regulating mitogenesis of smooth muscle cells, lipoprotein metabolism, and thrombogeneic response of endothelial cells, extracellular matrix production and

leukocyte adherence. This gene family have specific pathway for developing and rupturing of atherosclerotic plaque: (i) favor thrombosis by modifying endothelium, (ii) smooth muscles present in coronary artery are stimulated by the IL-1 gene through transforming growth factor-β (TGF-β) and (iii) it increases endothelial expression of adhesion molecules thus favoring atherosclerotic plaque progression.[56]

Interleukin-1B gene structure and SNP (-511) T>C

Interleukin gene 1 family is present on long arm of chromosome 2 present within 430-kb section of DNA. Interleukin 1 family contains nine genes including IL-1A, IL-1B, and IL-1RN.[58]IL-1β SNPC/T has a significant role in regulation of gene expression. The high levels of IL-1β expression is may be due to T (thymine) allele which replaced the cytosine in this single nucleotide polymorphism. Disease progression and severity depends upon the balance between IL-1 and IL-1Ra. There may occur an alteration in the Expression of IL-1receptor antagonist due to this single nucleotide polymorphism in the promoter region of IL-1β. Thus individuals carrying this polymorphism may changes susceptibility to diseases which includes interleukin 1 gene family such as myocardial infarction.[59] This also illustrates the variance in some ethnic region such as Caucasian have higher C allele frequency in control group as compared to cases while its opposite results are seen in African-Brazilians.[54] In Pakistan there is no available data related to this SNP so it is advantageous to study this polymorphism in this territory.

CONCLUSION

In conclusion, the complex diseases like CAD are difficult to dissect at the molecular level, however, advances in the understanding of the physiological pathways and the genes controlling the different biochemical pathways is helping to identify the risk factors unique to particular ethnic groups. In this regards, the role of common polymorphisms with low-modest effect sizes acting quantitatively can be crucial.

REFERENCES

1. Pranavchand R, Reddy B. Current status of understanding of the genetic etiology of coronary heart disease. J Postgrad Med2013;59(1):30-41.

2. Shrivastava AK, Singh HV, Raizada A, Singh SK. C-reactive protein, inflammation and coronary heart disease. Egypt Heart J2015;67(2):89-97.

3. Schelbert HR. Anatomy and physiology of coronary blood flow. J Nucl Cardiol 2010;17(4):545-54.

4. Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. Eur Heart J 2013;34(10):719-28.

5. Nabel EG, Braunwald E. A tale of coronary artery disease and myocardial infarction. NEnglJMed2012;366(1):54-63.

6. Kitsios GD, Dahabreh IJ, Trikalinos TA, Schmid CH, Huggins GS, Kent DM. Heterogeneity of the phenotypic definition of coronary artery disease and its impact on genetic association studies. CircCardiovascGenet2011;4(1):58-67.

7. Kazmi N, Gaunt TR. Diagnosis of coronary heart diseases using gene expression profiling; stable coronary artery disease, cardiac ischemia with and without myocardial necrosis. PloS one 2016;11(3):e0149475.

8. Hagström E, Norlund F, Stebbins A, Armstrong P, Chiswell K, Granger C, et al. Psychosocial stress and major cardiovascular events in patients with stable coronary heart disease. J Intern Med2018;283(1):83-92.

9. Khan HU, Khan MU, Noor MM, Hayat U, Alam MA. Coronary artery disease pattern: a comparison among different age groups. J Ayub Med Coll Abbottabad 2014;26(4):466-9.

10. Silbiger JJ, Stein R, Roy M, Nair MK, Cohen P, Shaffer J, et al. Coronary artery disease in South Asian immigrants living in New York City: angiographic findings and risk factor burdens. Ethn Dis2013;23(3):292-5.

11. Pradeepa R, Surendar J, Indulekha K, Chella S, Anjana RM, Mohan V. Prevalence of metabolic syndrome and its association with coronary artery disease among an urban elderly south Indian population (CURES-145). JAssocPhysicians India 2015;64(5):20-5.

12. Khan HS, Javed A, Aziz S, Ali J. Relationship between bmi and severity of coronary artery disease in female population of Pakistani origin. PakHeart J2012;44(1-2):4-7.

13. Hata J, Kiyohara Y. Epidemiology of stroke and coronary artery disease in Asia. Circulation2013;77(8):1923-32.

14. Hussain M, Khan N, Uddin M, Al Nozha MM. Chest pain, coronary artery disease and risk factors: a global snapshot. JDow UniHealth Sci2014;8(2):80-6.

15. Santulli G. Epidemiology of cardiovascular disease in the 21stcentury: updated numbers and updated facts. JCardiovasc Dis Res 2013;1(1):1-2.

16. Tan ST, Scott W, Panoulas V, Sehmi J, Zhang W, Scott J, et al. Coronary heart disease in Indian Asians. GlobCardiolSciPract2014:2014(1):13-23.

17. Naseem S, Khattak UK, Ghazanfar H, Irfan A. Prevalence of non-communicable diseases and their risk factors at a semi-urban community, Pakistan. Pan Afr Med J2016;23:151.

18. Habib S. Coronary artery disease in women. PakHeart J2011;44(1-2):18-26.

19. Krishnan M, Zachariah G, Venugopal K, Mohanan P, Harikrishnan S, Sanjay G, et al. Prevalence of coronary artery disease and its risk factors in Kerala, South India: a community-based cross-sectional study. BMC Cardiovasc Dis 2016;16(1):1-12.

20. Wasay M, Khatri IA, Kaul S. Stroke in South Asian countries. Nat RevNeurol2014;10(3):135-43.

21. Dodani S. Excess coronary artery disease risk in South Asian immigrants: can dysfunctional high-density lipoprotein explain increased risk? Vasc Health Risk Manag2008;4(5):953-61.

22. Sekhri T, Kanwar R, Wilfred R, Chugh P, Chhillar M, Aggarwal R, et al. Prevalence of risk factors for coronary artery disease in an urban Indian population. BMJ Open 2014;4(12):e005346.

23. Bhalli MA, Kayani AM, Samore NA. Frequency of risk factors in male patients with acute coronary syndrome. JCollPhysiciansSurgPak2011;21(5):271-5.

24. Nuri M, Nawaz A, Usman H. A proposed study of risk factors of heart disease in rural population of punjab (pakistan)--time to act. PakHeart J 2006;39(3-4):42-7.

25. Messner B, Bernhard D. Smoking and cardiovascular disease mechanisms of endothelial dysfunction and early atherogenesis. Arterioscler Thromb Vasc Biol2014;34(3):509-15.

26. Khan RJ, Harvey DJ, Leistikow BN, Haque KS, Stewart CP. Relationship between obesity and coronary heart disease among urban Bangladeshi men and women. Integr Obes Diabetes 2015;1(3):49-55.

27. Khan MS, Khan A, Ali A, Akhtar N, Rasool F, Khan H, et al. Prevalence of risk factors for coronary artery disease in Southern Punjab, Pakistan. TropJPharmRes2016;15(1):195-200.

28. Lavie CJ, McAuley PA, Church TS, Milani RV, Blair SN. Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. JAmCollCardiol2014;63(14):1345-54.

29. Muhammad R, Masood A, Zaffar J, Butt U. Correlation of mean hba1c levels with severity of coronary arteries disease in diabetics. PakHeart J2014;47(4):184-7.

30. Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J 2013;34(39):3035-87.

31. Sharma DS. Hypertension with special reference to causes and diet. Indian JApplRes2016;5(12):54-5.

32. Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. Journal Clin Invest2013;123(1):11-8.

33. Muñoz-Durango N, Fuentes CA, Castillo AE, González-Gómez LM, Vecchiola A, Fardella CE, et al. Role of the renin-angiotensin-aldosterone system beyond blood pressure regulation: molecular and cellular mechanisms involved in end-organ damage during arterial hypertension. Int J Mol Sci 2016;17(7):797.

34. van Vark LC, Bertrand M, Akkerhuis KM, Brugts JJ, Fox K, Mourad JJ, et al. Angiotensin-converting enzyme inhibitors reduce mortality in hypertension: a meta-analysis of randomized clinical trials of renin–angiotensin–aldosterone system inhibitors involving 158 998 patients. Eur Heart J 2012;33(16):2088-97.

35. Song C, Chang Z, Magnusson P, Ingelsson E, Pedersen N. Genetic factors may play a prominent role in the development of coronary heart disease dependent on important environmental factors. J Intern Med2014;275(6):631-9.

36. Attaur-Rasool S, Hasan S, Ghani N, Malik Z. Pattern of conventional risk factors in coronary artery disease patients. PakJPhysiol2013;9(2):19-22.

37. Maas A, Appelman Y. Gender differences in coronary heart disease. Netherlands Heart J 2010;18(12):598-603.

38. Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. ApplClinGenet 2014;7:15-32.

39. Buckley ML, Ramji DP. The influence of dysfunctional signaling and lipid homeostasis in mediating the inflammatory responses during atherosclerosis. BiochimBiophysActa 2015;1852(7):1498-510.

40. Jayashree S, Arindam M, Vijay KV. Genetic epidemiology of coronary artery disease: an Asian Indian perspective. J Genet2015;94(3):539-49.

41. Abelló D, Sancho E, Camps J, Joven J. Exploring the role of paraoxonases in the pathogenesis of coronary artery disease: a systematic review. Int J Mol Sci2014;15(11):20997-1010.

42. Macharia M, Hassan MS, Blackhurst D, Erasmus RT, Matsha TE. The growing importance of PON1 in cardiovascular health: a review. JCardiovascMed2012;13(7):443-53.

43. Swellam M, Mahmoud MS, Ali AA-F, Hefny MM, Hashim M. Clinical implications of PON1 gene polymorphism in patients with coronary artery disease. IntJToxicolPharmacolRes2016;8(1):7-12.

44. Agrawal S, Tripathi G, Prajnya R, Sinha N, Gilmour A, Bush L, et al. Paraoxonase 1 gene polymorphisms contribute to coronary artery disease risk among north Indians. Indian J Med Sci2009;63(8):335-44.

45. Kulka M. A review of paraoxonase 1 properties and diagnostic applications. PolJ VetSci2016;19(1):225-32.

46. Mackness M, Mackness B. Human paraoxonase-1 (PON1): gene structure and expression, promiscuous activities and multiple physiological roles. Gene 2015;567(1):12-21.

47. Eom SY, Kim YS, Lee CJ, Lee CH, Kim YD, Kim H. Effects of intronic and exonic polymorphisms of paraoxonase 1 (PON1) gene on serum PON1 activity in a Korean population. JKorean Med Sci 2011;26(6):720-5.

48. Morcos NYS, El-Masry MK, Kamal MM, Galal SY. Contrasting genetic influence of PON 1 coding gene polymorphisms L55M and Q192R on individuals' response to environmental agents. Egyptian JMedHumGenet 2015;16(3):233-7.

49. Martínez-Salazar MF, Almenares-López D, García-Jiménez S, Sánchez-Alemán MA, Juantorena-Ugás A, Ríos C, et al. Relationship between the paraoxonase (PON1) L55M and Q192R polymorphisms and obesity in a Mexican population: a pilot study. Genes Nutr 2011;6(4):361-8.

50. Deshpande CS, Singhal RS, Mukherjee MS. Association of paraoxonase1 gene Q192R polymorphism and apolipoprotein B in Asian Indian women with coronary artery disease risk. Genet Test Mol Biomarkers2013;17(2):140-6.

51. Ahmad I, Narang R, Venkatraman A, Das N. Two-and three-locus haplotypes of the paraoxonase (PON1) gene are associated with coronary artery disease in Asian Indians. Gene 2012;506(1):242-7.

52. Lao X, Wang X, Liu Y, Lu Y, Yang D, Liu M, et al. Association of paraoxonase 1 gene polymorphisms with the risk of hepatitis b virus-related liver diseases in a guangxi population: a case-control study. Medicine2015;94(48):   .

53. Lüersen K, Schmelzer C, Boesch-Saadatmandi C, Kohl C, Rimbach G, Döring F. Paraoxonase 1 polymorphism Q192R affects the pro-inflammatory cytokine TNF-alpha in healthy males. BMC Res Notes 2011;4(1):141.

54. Rios DL, Cerqueira CC, Bonfim-Silva R, Araújo LJ, Pereira JF, Gadelha SR, et al. Interleukin-1 beta and interleukin-6 gene polymorphism associations with angiographically assessed coronary artery disease in Brazilians. Cytokine 2010;50(3):292-6.

55. Eltoft A, Arntzen KA, Wilsgaard T, Mathiesen EB, Johnsen SH. Interleukin-6 is an independent predictor of progressive atherosclerosis in the carotid artery: the tromsø study. Atherosclerosis 2018;271:1-8.

56. Rai H, Sinha N, Kumar S, Sharma AK, Agrawal S. Interleukin-1 Gene cluster polymorphisms and their association with coronary artery disease: separate evidences from the largest case-control study amongst north Indians and an updated meta-analysis. PloS one. 2016;11(4):e0153480.

57. Tripathi G, Rangaswamy D, Borkar M, Prasad N, Sharma R, Sankhwar S, et al. Interleukin-1 gene cluster variants in hemodialysis patients with end stage renal disease: An association and meta-analysis. Indian JNephrol 2015;25(1):34.

58. Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. Am J Clin Nutr2006;83(2):475S-83S.

59. Coker A, Arman A, Soylu O, Tezel T, Yildirim A. Lack of association between IL-1 and IL-6 gene polymorphisms and myocardial infarction in Turkish population. Int J Immunogenet 2010;38(3):201-8.