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REVIEW ARTICLE

Molecular Studies on Coronary Artery Disease—A Review

A. Supriya Simon · T. Vijayakumar

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Abstract Coronary artery disease (CAD) remains the major cause of mortality and morbidity in the entire world population. The conventional risk factors of CAD include hypertension, hyperlipidemia, diabetes mellitus, family history, smoking etc. These factors contribute only 50 % of the total risk of CAD. For providing a complete risk assessment in CAD, it is mandatory to have well-planned clinical, biochemical and genetic studies in patients with CAD and subjects who are at risk of developing CAD. In this review an attempt is made to critically evaluate the conventional and emerging risk factors which predispose the individual to CAD. Specifically, the molecular basis of CAD including high oxidative stress, low antioxidant status and increased DNA damage are covered. A comprehensive and multifactorial approach to the problem is the better way to reduce the morbidity and mortality of the disease.

Keywords Coronary artery diseases (CAD) · DNA damage · Oxidative stress · Antioxidant status · Cytokinesis-block micronuclei (CBMN) assay

Introduction

Coronary artery disease (CAD) is the single most prominent disease entity in terms of both morbidity and mortality. Both men and women between the age group of 40 and 60 years are susceptible to it [1]. Despite all-round efforts in the prevention and management of this disease, it remains a major challenge to the health managers and scientists. It is predicted that by the year 2020, this disease would persist as the major and the most common threat to human life [2].

In developing countries, the incidence of CAD is increasing alarmingly. India is on the verge of a cardiovascular disease epidemic. In India, the incidence of CAD has more than doubled in the past two decades and predicted to be the main cause of death in the next decade [3]. In 1990, there were an estimated 1.17 million deaths from CAD in India, and the number has almost doubled to 2.03 million by 2010. In addition to the high rate of CAD mortality in the Indian subcontinent, CAD manifests almost 10 years earlier on average in this region compared with the rest of the world resulting in a considerable number of CAD deaths occurring in the working age group [4].

In Western countries, where CAD is considered a disease of the aged, 23 % of CAD deaths occur below the age of 70; this is in contrast with 52 % of CAD deaths occurring among people under 70 years of age in India. The Indian subcontinent suffers from an enormous loss of fruitful working years due to CAD and associated deaths. Estimated 9.2 million productive years of life were wasted in India in 2000, with an anticipated increase to 17.9 million years in 2030. The burden of CAD in the Indian subcontinent is the result of the large population and the raised prevalence of CAD risk factors [5]. Based on findings of the National Commission on Macroeconomics and Health (NCMH), there would be around 62 million patients with CAD by 2015 in India. Among these 23 million would be cases younger than 40 years of age [6]. Eighty percent of the heart attacks can be prevented by suitable management and

A. S. Simon (✉)
Department of Biochemistry, Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla 689 101, Kerala, India
e-mail: supriyasimon_a@yahoo.co.in

T. Vijayakumar
Educare Institute of Dental Sciences, Malappuram 676 504, Kerala, India

restriction strategies. Overall there is no program for the control of CAD in India, possibly on account of the major focus of the health policies on infectious disease and maternal & child health [7].

The cost of management of CAD is a significant economic burden and so prevention of CAD is very important in the management. Prevention of CAD can be approached in many ways including health promotion campaigns, specific protection strategies, life style modification programs, early detection and good control of risk factors and constant vigilance of emerging risk factors [8].

Although CAD is a fatal disease with no known cure it is a highly predictable, preventable, and treatable with the existing knowledge. CAD is a multifactorial process that appears to be due to the interaction of environmental risk factors with multiple predisposing genes [9]. Majority of patients who experience a CAD event have one or more of the conventional risk factors for atherosclerosis and so do many people who have not yet experienced such an event. The study of these risk factors is essential since the ability to predict precisely the CAD risk of a particular individual based on his or her conventional risk factor profile is limited [10]. Thus, predictive models based on conventional risk factors have lower than the desired accuracy, providing a stimulus to search for new factors to predict the risk of CAD [11]. For providing a complete risk assessment in CAD, it is mandatory to have well-planned clinical, biochemical and genetic studies in patients with CAD and subjects who are at risk of developing CAD [12]. Increased genetic propensity and increasing prevalence of cardiovascular risk factors are the reasons hypothesized for growing prevalence and greater severity and degree of CAD in Indians [13].

Many studies were conducted to evaluate the role of conventional risk factors like hypertension, hyperlipidemia, diabetes mellitus, family history, smoking etc. in CAD patients in India. These factors contribute only 50 % of the total risk of CAD [14]. This suggests that some major CAD risk factors are yet to be identified. Lipoprotein (a) [Lp (a)], homocysteine, higher oxidative stress, low levels of antioxidants, rising affluence, rapid modernization associated with sedentary but stressful life style are suggested as additional risk factors for CAD.

It has been proved that oxidative stress can cause chromosomal aberrations, extensive oxidative DNA damage and DNA strand breaks. DNA damage has been implicated as an important risk factor, which play a considerable role in atherosclerosis and carotid artery disease [15]. DNA damage is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus [16]. Usually the cells have repair mechanisms that identify and correct DNA damage. When the DNA repair mechanisms break down, a person may develop heart disease, brain deterioration and cancer. Therefore, DNA repair is essential to an individual's ability to respond to damage caused by environmental mutagens and reactive cellular metabolites [17]. Inter individual variability in DNA repair capability is an important factor influencing the risk of CAD. DNA repair gene polymorphism may also contribute to this variation [18].

Atherosclerosis is the most prevalent underlying cause of CAD, peripheral artery disease and carotid arterial disease. Atherosclerosis is a chronic immunoinflammatory, fibroproliferative disease of large and medium-sized arteries fuelled by lipid [19–21]. Atherosclerotic lesions consist of the cellular component containing mainly of smooth muscle cells and macrophages, the connective tissue matrix and extracellular lipid and the intracellular lipid that collects within macrophages, transforming them into foam cells. Atherosclerotic lesions evolve as a result of inflammatory stimuli, with consequent release of cytokines, synthesis of connective tissue matrix, proliferation of smooth muscle cells and collection of macrophages and lipids [22].

Conventional Risk Factors of CAD

Age

Coronary atherosclerosis starts in childhood and progresses with age [23]. CAD, specifically, coronary atherosclerosis occurs in about 5–9 % (based on race and sex) of people around 20 years. The mortality rate advances with age and overall is higher for men than in women, especially between the age span of 35 and 55 years. After age 55, the death rate for men falls and for women continues to rise. After age 70–75, the death rate for women surpasses that for men in the same age group. In an angiographic study in Malaysia, Asian Indians under the age of 40 years had a 15-fold higher rate of CAD, compared to Chinese and a tenfold higher rate in comparison to Malays [24]. About 25 % of acute myocardial infarction (MI) in India occurs under the age of 40 years and 50 % under the age of 50 years. MI was seen to develop 5–10 years in advance in Asian Indians than in other populations, and its incidence in patients under 40 years is fivefold to tenfold higher. The increased risk of CAD in Asian Indians seems to be greater at younger ages [25]. In a previous study by Simon et al. [26] the risk of CAD increased with progression of age.

Gender Differences

Numerous coronary angiographic studies exhibit lesser degrees of epicardial CAD among women than men of

similar age [27]. Even though women have a greater life expectancy than men and to exhibit CAD 10–20 years later than men, the onus of CAD in women is high, with a lifetime risk >20 %. Women have more beneficial lipoprotein profiles than men at the start of puberty. Levels of low-density lipoproteins (LDL) cholesterol and non-high-density lipoproteins (HDL) cholesterol are less in young and middle-aged women than in men of same age, but the opposite is true after menopause [28]. Lipoprotein levels fully predict incident and repeated CAD events in both sexes. LDL particle size may be a reliable predictor of early CAD in women than of CAD linked with advanced age. Non-HDL cholesterol appears to be a better measure of CAD risk in women than in men [29]. In an earlier study by Simon et al. [26] the high prevalence of CAD in males may be due to other risk factors such as smoking, alcohol consumption etc.

Family History

A positive family history of CAD is considered a risk factor for developing CAD [30]. First-degree relations of people who manifest CAD at an early age are at a greater chance for developing CAD than the usual population. Researchers have identified more than 250 genes that may play a role in CAD. It often ensues from the blended influences of multiple genes. These polygenic effects mean that the genetics of CAD are very complex, with many different genes influencing an individual's risk. Evidence of the strong genetic component for coronary heart disease risk is supported by the consistent association between a reported family history of early coronary heart disease and a personal increased risk [31].

Socioeconomic Status (SES)

Asian Indians with low SES have a greater prevalence of CAD and risk factors such as hypertension and smoking. However, a difference in SES does not explain the increasing burden of CAD among Asian Indians [32].

Urbanization

In urban India the prevalence of CAD is about double the rate in rural India. The rates appear to be higher in South India [33]. In spite of higher rates of smoking, CAD rates in rural India are half those in urban India [34].

Physical Inactivity

There is a direct association between physical inactivity and cardiovascular death, and physical inactivity is an independent risk factor for the development of CAD. Atherogenic risk factors are common in Asian Indians and worsen with weight gain [35]. Sedentary lifestyle is the most common risk factor for CAD in women. Physical inactivity will lead to obesity and this is a risk factor for myocardial infarction [36]. According to the World Health Organization, the lack of physical exercise contributes to approximately 17 % of diabetes and heart disease, 12 % of falls in elderly people, and 10 % of breast cancer and colon cancer [37].

Smoking

Smoking of cigarette is a significant modifiable risk factor for cardio vascular disease (CVD), including CAD, peripheral vascular disease, stroke and congestive heart failure [38]. The risk for CAD among smokers is related to dose, and the risk significantly increases with the number of cigarettes smoked per day. The increases in oxidation of LDL in persons who smoke promote monocyte adhesion and movement into the subintimal space. The continued stimulation of intimal cells by oxidized LDL leads to the advancement of atherosclerosis [39]. Tobacco smoke may cause insulin resistance, a risk factor for diabetes and CVD [40]. Smoking will increase the risk of all CVD; however, the magnitude of increased risk differs by CVD type [41]. The mechanisms by which cigarette smoke causes CVD are various and are synergistic. They include thrombosis, atherosclerosis, endothelial dysfunction and hemodynamic effects [42–44]. In a previous study by the authors [45] subjects having the habit of smoking have 10.035 times increased chance of developing CAD than subjects without smoking.

Alcoholism

Many prospective studies show an inverse association between moderate drinking and death from all cardiovascular causes [46]. Moderate alcohol consumption is associated with hypertension [47] and higher doses result in an increased risk of MI and stroke [48, 49]. According to Saremi and Arora, [50] there is an increased risk of hypertriglyceridemia, cardiomyopathy, hypertension, and stroke if 3 or more standard drinks of alcohol are taken per day.

Diabetes

The risk for CAD among diabetic subjects is greater by a factor of 2–4 compared to non-diabetic subjects [51, 52]. Emerging data indicate that even impaired glucose tolerance may predispose to progression of cardiovascular events and atherosclerosis [53]. These processes result in acute endothelial dysfunction in diabetic blood vessels,

which contribute overtly to the development of cardiovascular disease. CAD is the major cause of mortality and morbidity worldwide in type 2 diabetes, 65–80 % of deaths from type 2 diabetes are due to cardiovascular or cerebrovascular complications. The pathophysiology of type 2 diabetes is characterized by hyperglycemic spikes which induce oxidative stress. This along with soluble advanced glycation end products (AGEs) and lipid peroxidation products, are key activators of upstream kinases, which leads to endothelial dysfunction and expression of inflammatory genes [54].

Hypertension

Hypertensive patients are at increased risk to experience cardiovascular events throughout their life period and treatment of hypertension is one of the effective approaches to decrease global cardiovascular risk [55].Together with the other modifiable cardiovascular risk factors, such as hyperglycemia, smoking, obesity, and hypercholesterolemia, hypertension contributes to the world wide cardiovascular burden of morbidity and mortality [56]. The importance of reactive oxygen species (ROS) in vascular function and the development of hypertension have been recently reviewed [57, 58]. Increased vascular oxidative stress could be involved in the pathogenesis of hypertension, a major risk factor for cardiovascular disease mortality [59, 60]. In the previous studies by the authors [26, 45] hypertension is significantly associated with CAD.

Dyslipidemia

Dyslipidemia has long been shown to have a strong relation with CAD. According to NCEP—ATP III (National Cholesterol Education Program—Adult Treatment Panel), hypercholesterolemia Guidelines [61], hypercholesterolemia is defined as total cholesterol >200 mg/dl, LDL as >100 mg/dl, HDL <40 mg/dl and hypertriglyceridemia as TG >150 mg/dl. Dyslipidemia is defined by the presence of one or more abnormal serum lipid concentration. Disorders of plasma lipoprotein metabolism (“dyslipidemia”) play a major role in the initiation and progression of atherosclerotic cardiovascular disease [62].

Low-density lipoproteins (LDL), and other atherogenic apoB-containing lipoproteins, are known to promote cholesterol collection in macrophages as well as inflammatory responses inside the vessel wall, leading to atherosclerosis progression. Lipid deposition starts with the mobility of LDL from the blood into the wall of the vessel. Once within the media of the blood vessels three outcomes can occur: it may move back into the bloodstream (a sign of lesional regression and a process that may be expedited by some lipid reducing strategies), it may become oxidized (through direct activity of leukocytes or action of free radicals) or it may be taken up by monocyte/macrophages which eventually become foam cells. Oxidized LDL is especially atherogenic and is chemotactic for monocyte-macrophages [22].

Oxidative modification of LDL is brought about by free radicals, which cause degradation of polyunsaturated fatty acids and the development of lysolecithin, oxysterols and aldehyde alteration of lysine residues on apoB [63]. Oxidation of LDL leads to many changes in the composition of the particle, which vary depending on the concentration and type of oxidant used and the duration of exposure. Depending on the degree of oxidation, in ‘minimally’, lightly or mildly Ox-LDL (oxidized LDL), apoB is modified only to a minor extent and is still able to bind to the LDL receptor and is taken up via clathrin coated pits while in heavily Ox-LDL, apoB prevent LDL from binding to LDL receptors, and instead Ox-LDL is recognized by receptors such as scavenger receptor A (SR-A) and CD36 [63, 64]. Small dense LDL is more toxic or atherogenic to the endothelium. Ox-LDL is directly chemotactic for monocytes and T cells (not for neutrophils or B cells, neither of which are found in lesions). Ox-LDL promotes recruitment and retention of monocytes with the formation of fatty streaks, the earliest lesions in atherosclerosis.

The strong connection between low levels of HDL and the risk for atherosclerosis and CAD has been credited to several distinct mechanisms.HDL and its major apo lipoprotein, apoA-I, promote the efflux of cholesterol from macrophages, the first step in the process of reverse cholesterol transport, and have other anti-inflammatory, antithrombotic and antioxidant effects that may contribute to inhibition of atherosclerosis. Human and animal studies have raised the possibility that HDL also slows vascular disease by blocking inflammation [65]. An atherogenic lipoprotein pattern, distinguished by a predominance of small dense LDL, moderately elevated plasma triglycerides and low HDL levels, is the most important risk factor for CAD [66].

Elevated plasma triglyceride (TG) concentration is becoming increasingly established as a risk factor for premature CAD [67]. The contributing factor for hypertriglyceridemia in our population could be our diet rich in carbohydrates. In a previous study by Simon et al. [26], elevated LDL cholesterol and triglyceride and decreased HDL cholesterol was observed in CAD patients.

Obesity

Obesity is more frequently identified as an epidemic and a modifiable risk factor for CAD [68]. Obesity is associated with increased risk of hypertension, diabetes, dyslipidemia, and CAD. Earlier reports have documented that increased

cardiovascular (CV) risk associated with being overweight can be explained by its association with various risk mediators, including traditional atherosclerotic risk factors, inflammation, insulin resistance, and endothelial dysfunction [69].

Abdominal Obesity

Abdominal obesity represents a central component of cardio metabolic risk, one that is mechanistically linked to many other individual risk factors [70]. Visceral (abdominal) fat is a strong predictor of dyslipidemia, glucose intolerance, insulin resistance, systemic inflammation, as well as incidence of hypertension, cardiovascular disease, Type 2 Diabetes mellitus, and causes mortality, independent of other fat depots. Additionally, the reduction of visceral fat through lifestyle intervention is associated with improvements in cardio metabolic risk factors independent of changes in other fat depots [71].

Emerging Risk Factors

Despite the long list of traditional and conventional risk factors, 50 % of the CAD still remains unexplained. This led researchers to think of novel risk factors, which might contribute to CAD. Some of the comparative studies on migrant Indians have suggested that the excess risk for CAD seen among Indians could be partly explained by these risk factors [72, 73]. Some of the newer risk factors such as lipoprotein (a), homocysteine, coagulation and fibrinolytic factors, inflammatory markers and high-sensitivity C-reactive protein (hs-CRP) are linked with higher risk for CAD. DNA damage is increasingly recognized as being present in all cells within the atherosclerotic plaque and DNA damage may promote atherogenesis. DNA damage has been found as an emerging risk factor in atherosclerosis and CAD [15].

Oxidative Stress

Oxidative stress is a large rise in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage [74, 75]. Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes. One of the major toxic effects of excessive ROS is damage to cellular membranes by the process of lipid peroxidation. Preferential targets for chemical reactions are double bonds; e.g. in PUFAs or guanine bases in DNA [76].

Reactive oxygen species cause structural alterations in DNA, e.g. base pair mutations, rearrangements, deletions, insertions and sequence amplification. $OH^{\bullet}$ is especially damaging, but $O_2^{\bullet -}$ $RO^{\bullet}$, $RO_2^{\bullet}$, HNO_2,O_3, ONOO− and the decomposition products of ONOO− are also effective. The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination and deamination [77, 78]. ROS may have some deleterious effects on DNA and can provoke extensive oxidative DNA damage, DNA strand breaks, and chromosomal aberrations [79]. Nitric oxide or, more likely, reactive products derived from it, such as $NO_2^{\bullet}$, ONOO−, N_2O_3 and HNO_2, are mutagenic agents, with the potential to produce nitration, nitrosation and deamination reactions on DNA bases. Besides these direct effects of oxidative injury, there can be indirect injury because the nicks and breaks in DNA strands can trigger activation of Poly (ADP) polymerase (PARP), which alters gene expression, DNA replication and may trigger apoptosis. It can also deplete NAD+, which leads to cellular ATP depletion [80].

Many recent studies have demonstrated an association between increased oxidative stress and diabetes, hypertension, cigarette smoking and dyslipidemia, which are well known risk factors for atherosclerosis. Oxidative stress both promotes and is induced by vascular diseases and risk factors that lead to vascular disease [75]. ROS are the most likely agents inducing DNA damage in atherosclerosis [81]. A crucial step in the pathogenesis of atherosclerosis is believed to be the oxidative modification of low density lipoprotein (LDL). The effects of lipid peroxides i.e. endothelial cell damage, uncontrolled lipid uptake, decreased prostaglandin synthesis and associated thrombogenicity are strongly implicated in the pathogenesis of atherosclerosis. Further products generated in lipid peroxidation are aldehydes. The reaction of aldehydes with amine groups of peptides and proteins has been suggested as a mechanism involved in the modification of lipoproteins. The oxidation of LDL is a free radical driven lipid peroxidation process and the aldehyde products of lipid hydroperoxide breakdown are responsible for the modification of the LDL apoprotein. Aldehyde-modified apoB protein has altered receptor affinity, causing it to be scavenged by macrophages in an uncontrolled manner with the development of foam cells and the initiation of the atherosclerotic lesion. The aldehydic products of lipid peroxidation may also be involved in other aspects of the development of the lesion. The oxidation of LDL may be prevented by its endogenous antioxidant compounds, most prominent of which is α-tocopherol [82].

Many studies have linked excess generation of ROS with cellular damage and atherogenesis. A growing body of

evidence indicates that oxidative DNA damage is also a prominent feature of atherosclerotic plaques [79]. ROS are involved in oxidation of LDL, which is considered as a fundamental step in the initiation and progression of atherosclerosis [83]. Oxidative stress ensues when ROS evade or overwhelm antioxidants [84]. There is an increased oxidative stress in CAD due to the excessive production of ROS, which may result in reduced total antioxidant capacity. This results in an imbalance between antioxidant power and prooxidants, which can facilitate and augment the atherosclerotic process [75]. ROS normally exist in all aerobic cells in balance with biochemical antioxidants [85].

Malondialdehyde is a decomposition product of autoxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. Many studies have found increased MDA levels in the CAD patients compared to normal [1, 63, 84, 86, 87]. The relationship between oxidative stress parameters and inflammatory species suggest their strong mutual involvement in the development of atherosclerosis that leads to CAD [88].

Antioxidant System

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [89]. Since we live in an oxygen rich environment, and ROS are byproducts of normal metabolism, potent protective mechanisms have evolved to allow life to continue. The ROS decrease the antioxidant capacity or inhibit the antioxidant enzyme activity culminating in toxicant induced oxidative stress [90].

Antioxidants are exogenous or endogenous compounds acting in several ways, scavenging ROS or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation. Enhanced lipid peroxidation may occur as a result of the fact that, naturally occurring scavenging mechanisms are suppressed and the free radical generation processes are enhanced [91, 92]. Recently, increased oxidative stress and impaired anti oxidant defense have been suggested as a contributory factor for initiation and progression of complications in CADs [93].

In the vascular system, the formation of ROS from endothelial cells, smooth muscle cells and macrophages may be of major relevance in atherogenesis. Under normal conditions, numerous cellular antioxidant systems exist to defend against oxidant stress and maintain the redox balance of the cell. Antioxidants exert their protective effect against cardiovascular diseases by two fundamental mechanisms. The first mechanism is LDL—specific antioxidant action i.e. the protection of LDL against oxidative modification by antioxidants present either in LDL or in the extracellular fluid of the sub endothelial space. The second mechanism of antioxidant action is tissue or cell specific i.e. increased uptake of antioxidants by vascular cells and increased cellular antioxidant status. This increase in cellular antioxidants may result in decreased production or release of ROS and thus less cell-mediated LDL oxidation. In addition, the cellular antioxidants may increase the resistance of vascular cells to the damaging effects of modified LDL. Both the LDL-specific and cell-specific antioxidant actions may lead to decreased adhesion molecule and monocyte chemotactic protein-I (MCP-I) expression, decreased foam cell formation, and increased nitric oxide activity, and thus improved vascular function and decreased atherogenesis [63]. Antioxidants tend to reduce the risk and severity of atherosclerosis by inhibiting lipid peroxidation. This scenario suggests that together with the estimation of plasma lipids and lipoproteins, evaluation of oxidant–antioxidant profile in an individual will contribute significantly to the risk assessment, prophylaxis and management of CAD.

Glutathione is the most abundant intracellular thiol-based antioxidant, prevalent in millimolar concentrations in all living aerobic cells, and plays an important role in the cellular defense cascade against oxidative injury [94]. In addition to GSH, the liver also contains other antioxidants with low molecular weight such as vitamin C, vitamin E, ubiquinol, carotenoids, and bilirubin. They function in different subcellular compartments depending on their hydrophilic or hydrophobic nature. In contrast, vitamin E and other hydrophobic antioxidants function predominantly in and around membrane/lipid bilayers [95]. Kaur et al. [84] found a significant decrease in whole blood glutathione levels in all the subgroups with CAD as compared to control group. In a previous study by the authors revealed a significant correlation between CAD and GSH level [87].

Ascorbic acid is a water-soluble antioxidant that acts as the body's primary defense against peroxyl radicals formed in the aqueous phase Ascorbic acid is a reducing agent and can reduce, and thereby neutralize, ROS such as hydrogen peroxide [96]. It is the only antioxidant in plasma capable of completely inhibiting oxidative modification of LDL by aqueous peroxyl radicals [97]. Many studies documented significantly lower levels of vitamin C in CAD patients compared to the normal control groups. [63, 87, 97–99].

DNA Damage

The most prominent theory concerning the pathophysiological mechanisms of atherosclerotic plaque formation is the "inflammatory response to injury" hypothesis, which states that smooth muscle cells (SMC) proliferation is an inflammative-fibroproliferative reaction to various insults to the artery wall. However, some evidence suggests that alterations at the DNA level may contribute significantly to the development of the disease. In accordance with these findings, the "monoclonal" hypothesis of atherosclerosis has been suggested. This hypothesis proposes that atherosclerosis begins as a mutation, transforming a single, isolated smooth muscle cell into the progenitor of a proliferative clone, as seen in carcinogenesis.

The "monoclonal" hypothesis of atherosclerosis was first proposed by Benditt and Benditt [100] after analyzing the isoenzyme pattern in lesions from women heterozygous for an X-linked marker enzyme, glucose-6-phosphate-dehydrogenase (G6PD). This study demonstrated that all of the cells in an atherosclerotic lesion usually express only one specific allele, suggesting that plaque formation results from a somatic mutational event in a single smooth muscle cell that gives it and its progeny proliferative advantage, thereby producing a plaque. More extensive evidence for plaque monoclonality has recently come from a method based on polymerase chain reaction (PCR) amplification of the DNA of an X-inactivated gene [101]. Like the process of human cancer development, plaque formation may be a response to a somatic mutation that permits the expansion of the mutated cells. According to "monoclonal" hypothesis the plaque may be regarded as a monoclonal benign neoplasm of the artery wall [102]. A number of studies have showed that both cancer and atherosclerosis are characterized by a local increase in tissue mass that may be hard to control, and appears that the disease state of atherosclerosis and cancer might share a common etiology [103].

A similar hypothesis was suggested by Trosko and Chang [104], that somatic cell mutations were involved not only in the etiology of cancer but also in that of atherosclerosis and diabetes. Mutant cells might have a selective advantage over their sister cells in their ability either to replicate or to survive [105]. In accordance with the clonal hypothesis, a complex series of independent molecular events would have to occur in plaque cells, such as the accumulation of damage in crucial regions of DNA. If these lesions are not repaired (or not repaired with fidelity) they can be implicated in the initiation and/or progression of atherosclerosis and transmitted to daughter cells in the developing plaque. DNA changes in a cell may result from a spontaneous mutation, an inherited gene defect, or environmental insults, such as inhaled cigarette smoke, chemical mutagens, or viral infection. If the mutations occur in genes responsible for carcinogenesis (e.g., proto-oncogenes or tumor-suppressor genes) they may contribute to tumor genesis. The idea that such genes are involved in cardiovascular disease arose from the observation that the c-H-ras proto-oncogene is overexpressed in rat aortic smooth muscle cells after benzo [a] pyrene exposure [106].

DNA samples extracted from smooth muscle cells possess transforming ability when transfected into 3T3 cells; the transformed cells were able to induce tumors in nude mice [107]. Moreover, the p53 protein, which exhibits tumor suppressor activity, is abnormally accumulated in smooth cells of some patients who undergo a coronary angioplasty with a consequent enhanced myocell proliferation, called restenosis [108].

Molecular and cytogenetic studies indicate that chromosomal alterations are involved in a range of disorders and pathophysiological conditions related to atherosclerotic lesions. Cytogenetic assays in primary cultures of human atherosclerotic plaques have shown a monoclonal expansion of cells with chromosomal alterations, of which the loss of the Y chromosome and trisomy 7 were most common [109]. The presence of an extra chromosome 7 could be correlated with over expression of the gene for chain A of the platelet-derived growth factor (PDGF) with a consequent increase in the proliferative activity of smooth muscle cells [109]. Moreover, in aortic endothelial cells of aging subjects, as well as of patients with atherosclerosis, a high level of aneuploidy was found [110]. These aneuploid cells also shared an increased uptake of LDL cholesterol in sub endothelial intima. Fossel [111] suggested an important role for telomere shortening in atherogenesis. These data suggest that an alteration of telomere length and/or telomerase activity may be a pivotal event leading to the transformation of smooth muscle cells in a proliferative clone in atherosclerotic tissues.

Important evidence demonstrating specific molecular alterations in the atherosclerotic lesions has been obtained from microsatellite sequences. Microsatellites consist of short, highly repetitive DNA sequences that are prone to replication errors, in particular frame shift mutations. In a small fraction of cancers, mainly sporadic human tumors, defective repair of mismatched bases results in an increased mutation rate and consequent widespread microsatellite instability [112]. Mc Caffrey et al. [113] found an increased mutation rate of microsatellite sequences in patients with cardiovascular atherosclerotic lesions. The demonstration of microsatellite instability in human atherosclerotic plaques suggests that genomic destabilization, which may also affect other genes, can result in the misregulation of the cells harboring these mutations and may play a pivotal role in atherosclerotic mechanisms. A cell clone with microsatellite instability

probably acquires a proliferative advantage, leading to growth of the smooth muscle cells specific for the atherosclerotic plaque. It is noteworthy that microsatellite sequences were mutated in the atherosclerotic tissues but not in normal vascular tissues from the same patients. The observations indicate that there is a common pattern of microsatellite sequence mutation in atherosclerotic plaque collected from different patients and from experimental animals [113, 114].

A pronounced increase in mortality from cancer as well as atherosclerosis-related disease has been reported for populations exposed to arsenic and dioxin. Epidemiological studies indicate that certain carcinogenic agents, including vinyl chloride monomer (VCM) and industrial combustion effluents containing polycyclic aromatic hydrocarbons (PAHs) do have atherogenic effects [115, 116]. Finally, previous exposure to ionizing radiation seems to correlate with premature and localized severe plaque formation in the irradiated area [117]. Assuming that atherosclerotic and neoplastic lesions have similar pathogenetic mechanisms, it could be expected that at least some well-known mutagenic agents would exert atherogenic as well as carcinogenic effects and that populations at high risk for cancer may show an increased incidence of atherosclerosis-related diseases [103].

A series of molecular markers and gene-regulating pathways have been associated with disease development and progression common in both atherosclerosis and cancer [103]. These chronic diseases appear to be multi-staged in their progression, with genetic, nutritional, psycho-social, environmental and viral factors influencing their appearances. In addition, the experimental and clinical studies on atherosclerosis and cancer have also showed common pathogenic mechanisms of clotting system. Furthermore, emerging novel therapeutic strategies have similarly targeted both atherosclerosis and cancer, including reducing oxidative stress, inhibiting chemokine, cytokine, and growth factor cell signal transmit, down-regulating excess matrix digestion, inactivating nuclear factor-κ B (NF-κ B) signal pathway, interfering cell cycle regulation, applying radiation treatment for controlling expansion and invasion of both atherosclerosis and cancer. Taken as a whole, these findings suggest that atherogenesis is associated with mutagenesis and carcinogenesis [103].

Botto et al. [118] and Andreassi [16] reported that patients with CAD have a higher micronucleus index (a marker of genetic instability) than healthy controls which correlates with disease severity. Furthermore, the use of accepted biomarkers of carcinogenic exposure-such as DNA adducts and cytogenetic end points-recently has provided evidence consistent with the view that somatic cell alterations are critical in atherogenic process [119].

It follows that the study of DNA damage may provide new insights into the pathogenesis of atherosclerosis and lead to the development of novel therapeutic approaches [114]. Growing evidence indicates that oxidative damage to DNA may represent an important link between the inflammatory nature and the oxidative theory of atherosclerosis. Various animal models of atherosclerosis support the evidence that oxidatively damaged DNA plays a key role in both the formation and the complications of atherosclerosis. Human investigations also support a mutational hypothesis of atherosclerosis [120]. It is accepted that increased levels of DNA damage induced by xenobiotics play an important role in the early phases of atherogenesis [121]. Studies of DNA damage and mutagenesis, both genomic and mitochondrial, in atherosclerotic and vascular lesions, have yielded evidence that somatic mutations are involved in atherogenesis and vascular disease development [122].

DNA damage produces a variety of responses, including cell senescence, apoptosis and DNA repair. Such damage, if left unrepaired, can cause mutations, which can lead to disease. To protect against genome instability and its dangerous consequences organisms have developed several DNA repair pathways [123].

Somatic damages are detected via chromatin loss from the nucleus leading to micronuclei (MN) in the cytoplasm of the cell. MN is scored by Cytokinesis-Block Micronuclei (CBMN) Assay developed by Fenech [124]. According to Demirbag et al. [125] and Satoh et al. [126] DNA damage is increased in patients with metabolic syndrome. An earlier study by Simon et al. [26] also shown that the CBMN frequency of the CAD patients was significantly higher compared to their normal counter parts indicates an increase in DNA damage. The increase in DNA damage might be occurring because of the increase in the imbalance between the production of oxidants and antioxidant defenses in subjects with metabolic syndrome. Metabolic syndrome induces an increase in oxidative stress and may be an important contributory factor for CAD.

In a study on cardiovascular autonomic neuropathy (CAN) by Simon et al. [45] a strong correlation between CBMN frequencies and cardiovascular autonomic neuropathy was observed. The CBMN frequency was found to be high with life style factors like physical activity, smoking and alcoholism and risk factors like diabetes mellitus and dyslipidemia.

Conclusion

From these studies it can be concluded that CAD is the result of multifactorial influences, some are modifiable and others are not. The increase in the prevalence, morbidity and mortality can be strongly attributed to the changes in lifestyle habits along with the habit of smoking and/or

alcoholism. Diabetes mellitus and hypertension are the major risk factors for CAD. These risk factors are associated with an increased oxidative stress which are the most likely agents inducing DNA damage in atherosclerosis. So that the prevalence, morbidity and mortality of the disease can be reduced drastically by direct intervention in the form of lifestyle modifications such as increasing physical activity, decreasing intake of oil as well as avoidance of smoking and alcoholism. Adequate glycemic and blood pressure control in diabetics and hypertensives will reduce the risk of CAD. Higher intake of natural antioxidants will be a protective factor. These lifestyle modifications will reduce the risk of CAD by reducing DNA damages and improving DNA repair pro-efficiency.

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