CHAPTER II REVIEW OF LITERATURE

This section provides a selective review of pathophysiology of atherosclerosis, traditional risk factors, non-traditional risk factors or markers (hsCRP, fibrinogen, TNF-α, IL-6, Lp(a) and apolipoproteins) and pleiotropic effects of statins especially, atorvastatin.

2.1. Pathophysiology of atherosclerosis

Atherosclerotic vascular disease is the reason for the common chronic arterial diseases such as peripheral arterial disease, CHD and stroke leading to reduction in vascular reserve. Advanced atherosclerosis is directly related to cause thrombotic complications such as unstable angina, myocardial infarction (MI) and stroke (Libby and Ridker, 2004). The complex process of atherosclerosis involves numerous pathogenic cellular mechanisms, such as endothelial dysfunction, inflammation, accumulation of oxidatively modified lipids and matrix components in the arterial vessel wall, vascular smooth muscle cell (VSMC) activation and apoptosis, vascular remodelling, thus leading to plaque formation and progression (Libby, 2002).

This process can be activated and promoted by a number of risk factors, such as diabetes mellitus, obesity, hypercholesterolemia, hypertension and cigarette smoking. Both clinical and animal studies show that dyslipidemia causes atheroma formation. During early phase of atherogenesis, addition of excess oxidatively modified low-density lipoprotein (oxLDL) in atheroma caused by hypercholesterolemia can trigger endothelial cell (EC) dysfunction and leukocyte invasion into the arterial wall (Steinberg et al., 1989). Atherosclerotic plaque development begins with activation of arterial ECs and more expression of leukocyte adhesion proteins such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), promoting leukocyte attachment (Libby, 2002). The expression of monocyte chemoattractant protein-1 and -3 (MCP-1 and MCP-3) are increased and it enhances the migration of monocytes into the intima of the blood vessel wall.

These atheroma located macrophages express scavenger receptors to overwhelm and accumulate lipoproteins (e.g. oxLDL), thus transforming into foam cells, a distinguish characteristic during early atherosclerosis. This latter secrete numerous pro-atherogenic factors, such as TNF-α, macrophage colony-stimulating factor (M-CSF), interleukin-1β (IL-1β),
matrixmetalloprotease-2 and -9 (MMP-2 and MMP-9) and release reactive oxygen species (ROS) and all these contributes the accumulation of lipid in atherosclerotic lesions. Other significant sources of ROS are vascular VSMC, ECs and fibroblasts. The characterized effects of ROS include oxidation of LDL cholesterol and scavenging of endothelial produced nitric oxide (NO), in addition DNA damage and increased production of inflammatory transcription factors (Libby, 2002).

This oxidative stress promotes expression of VCAM-1, MCP-1 and MCP-3 on ECs and decreased endothelial NO-synthase (eNOS) expression. These effects consequently favour smooth muscle cell (SMC) proliferation and migration into the neointima, remodeling of the extracellular matrix (ECM) and monocyte invasion. ECs play a significant role by secretion of paracrine and autocrine factors beside NO, such as prostaglandins, angiotensin II, metalloproteinases (MMPs) and endothelin. This leads to the degradation of ECM structures, thus reducing plaque stability and promoting plaque rupture, a late complication of atherosclerosis. The activated VSMCs are generally responsible for production of large amounts of ECM proteins including collagen, elastin, fibronectin, proteoglycans, laminin, thrombospondin and vitronectin (Libby, 2002).

When the plaque enlarges up to >40% of the vessel area, the artery ceases enlargement and the lumen become narrows as the plaque is growing. Following lesions development, endothelial dysfunction appears to support progression of clinical processes. These vulnerable plaques are distinguished by a large lipid core, a thin fibrous cap and infiltrated macrophages at the surface of the cap and gathering of apoptotic VSMCs, driven into apoptosis by Fas/FasL mediated pathway (Boyle, 2005) and over expression of growth factors (Cicha et al., 2005). The inflammatory mediators found in atheroma include IL-1β, TNF-α and CD154, augmenting MMP expression. MMPs mediates the degradation of the cap, which are formed by activated SMC, ECs and macrophages, culminating in plaque rupture, thus releasing the lipid core including tissue factor and this further attracting and activating the circulating platelets, which adhere rapidly to the vessel wall.

Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) are secreted by the activated platelets and, of which latter is a potent stimulus for VSMC-mediated
collagen synthesis, promoting the growth of plaque. The rupture of plaque favours the occlusive thrombosis formation (Davies, 1996) and arterio-arterial platelet embolism. Patients affected by intracranial atherosclerosis have an elevated risk for recurrent major vascular events such as TIA and stroke.

2.2. Traditional risk factors

Although earliest research has recognized hypertension, diabetes and hypercholesterolemia as traditional CVD risk factors, several researchers have reported their absence in a considerable portion of individuals experience clinical vascular events. Indeed, up to half of those having their first clinical vascular events does not have traditional CVD risk factors (Braunwald, 1997). However, these findings may not be relevant to all populations, researchers from the FHS report that 50% of the patients with CHD had levels of TC ≤240 mg/dl and 20% had TC <200 mg/dl (Kannel, 1995).

Data from the Women’s Health Study (WHS) confirmed those three quarters of coronary events happen in 27,939 women without a high level of LDL cholesterol (<160 mg/dl) and 45% happen in women with normal LDL cholesterol (<130 mg/dl) (Ridker et al., 2002). When numerous large studies of CVD were reviewed, as one would expect, most individuals had one or more traditional risk factors (Khot et al., 2003). Conversely, one fifth had none of the traditional risk factors. In addition, among cohort individuals who did not suffered CHD, the rates of traditional cardiovascular risk factors were also relatively high, sustaining the idea that there must be other factors influencing the development of CVD (Greenland et al., 2003). Given these findings, new research has focused on ways of enhancing our ability to predict CVD. However, many of these show promise and most widely used in routine clinical practice.

2.2.1. Hypertension and cardiovascular disease

The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (1997) defines categorical hypertension as a BP ≥140 mmHg systolic or ≥90 mmHg diastolic or current use of antihypertensive prescription. Several observational studies have confirmed clearly a powerful relationship between high BP and CHD (Stamler et al., 1993; Franklin et al., 1999; van den Hoogen et al., 2000). This relationship holds for both men and women and younger and older persons. Occasionally below categorical
hypertension, subjects with high-normal BP are at increased risk for CHD compared with those with optimal values (Rodgers and MacMahon, 1999).

2.2.2. Diabetes and cardiovascular disease

Risk for all forms of CVD, is increased significantly patients with type 1 and type 2 diabetes mellitus (Wingard and Barrett-Connor, 1995; Bierman, 1992). The mortality rate in diabetic patients who have experienced CHD is much higher than in non-diabetic subjects (Herlitz et al., 1992; Miettinen et al., 1998). The increase in risk attributed to hyperglycemia as such is independent of the overweight/obesity and dyslipidemia commonly observed in persons with diabetes. Good glycemic control decreases risk for microvascular complications of diabetes. However, improved glucose control in diabetic people has not been definitively shown to reduce macrovascular disease (CHD), although a trend toward benefit has been observed (Diabetes Control and Complications Trial Research, Group, 1993).

2.2.3. Total cholesterol, low-density lipoprotein cholesterol and cardiovascular disease

Cholesterol is synthesized almost in all cells and considerable amounts of it can be absorbed from the diet. According to the lipid hypothesis, unusually high cholesterol levels (hypercholesterolemia), or higher concentrations of LDL cholesterol have been recognized as principle lipid risk factors (Durrington, 2003). Various studies have confirmed that blood TC levels have an exponential effect on cardiovascular and total mortality, with the association more evident in younger subjects. The impact of high cholesterol on health is still larger in older people (Lewington et al., 2007).

Several studies have consistently confirmed a dose-response relation between TC level and CHD risk. The Multiple Risk Factor Intervention Trial (MRFIT) screened >300,000 men and established a curvilinear relation between TC and age-adjusted CHD death rate; in MRFIT screeners with a TC level of ≥240 mg/dL, relative risk (RR) for CHD death was 3.4 compared with those with TC <182 mg/dL (Neaton and Wentworth, 1992). Conversely, the influence of factors other than TC on CHD risk is clearly established by the findings of 25 years of follow-up in the Seven Countries Study (SCS) (Verschuren et al., 1995), in which a dose-response
association was observed between TC and CHD mortality rate, yet at any given level of TC, CHD mortality rates varied by 4- to 5-folds.

Studies across different populations demonstrate that those with higher cholesterol levels have more atherosclerosis and CHD than do those who having lower levels (Keys et al., 1984). The positive association between serum cholesterol levels and the development of first or subsequent attacks of CHD is observed over a broad range of LDL cholesterol levels; the higher the level, the greater the risk (Stamler et al., 1986). Prospective data recommended that the risk of CHD at lower cholesterol levels and this evident has disappeared in larger studies (Stamler et al., 1986; Law, 1999). Only in populations that maintain very low levels of serum cholesterol, e.g., TC <150 mg/dL (or LDL cholesterol <100 mg/dL) throughout life do we find a near-absence of clinical CHD (People’s Republic of China-United States Cardiovascular and Cardiopulmonary Epidemiology Research Group, 1992; Law et al., 1994).

The association between the elevated LDL cholesterol to the development of CHD must be viewed as a multi-step process (Stary et al., 1995). In the first stage of atherogenesis is the fatty streak, which consists mainly of cholesterol-filled macrophages; most of the cholesterol in fatty streaks which derived from LDL cholesterol. In second stage the fibrous plaques in which a layer of scar tissue overlies a lipid rich core. Other risk factors also contribute to plaque growth. The third stage is demonstrated the development of unstable plaques that are prone to rupture and formation of luminal thrombosis. Plaque rupture is responsible for most acute coronary syndromes (ACS) (Libby et al., 1998; Fuster et al., 1999).

2.2.4. Triglycerides, very low-density lipoprotein cholesterol and cardiovascular disease

TG is a ester formed from a glycerol molecule, combined with three fatty acids on each of the OH groups and make up the majority of fats digested by humans. Lipids cannot be absorbed by the duodenum in TG form and it is absorbed as fatty acids, monoglycerides and some diglycerides, once the TG have been digested. In the human body, high levels of TG in the bloodstream have been linked to atherosclerosis and CHD.

Several observational studies and analyses published in the earlier years largely support TG as an independent risk factor for CHD. These studies have been conducted in populations
across a wide spectrum of ages in a number of countries with quite different rates of CVD (Austin et al., 1998; Avins and Neuhaus, 2000; Simons et al., 2001; Sharett et al., 2001). Traditionally, elevated TG has predicted CHD events in univariate analysis, after adjustment for other covariates, including plasma glucose and HDL cholesterol, to which it is strongly and inversely correlated (Criqui et al., 1993). Yet, even after adjustment for HDL cholesterol, detailed assessment of population-based prospective studies has disclosed an independent effect of TG on CHD events (Sarwar et al., 2007). Coupled with the knowledge that combined hyperlipidemia promotes CHD to a significantly greater extent than either high LDL cholesterol or TG alone (Manninen et al., 1992).

Very low-density lipoprotein (VLDL) cholesterol is a type of lipoprotein formed by the liver, which enable fats and cholesterol to move within the water based solution of the blood stream. It is accumulated in the liver from cholesterol and apolipoproteins, which converted in the bloodstream to LDL cholesterol. VLDL cholesterol transports endogenous products (such as TG, phospholipids, cholesterol and cholesteryl esters) where chylomicrons transport exogenous (dietary) products.

The most likely candidates for atherogenic TG-rich lipoproteins (TGRLP) are remnant lipoproteins. These lipoproteins include small VLDL cholesterol and intermediate-density lipoproteins (IDL). They are cholesterol-enriched particles and have many of the properties of LDL cholesterol. Reviews of several independent lines of evidence support the atherogenicity of remnants (Havel, 1990; Grundy, 1998; Krauss, 1998). In several clinical studies in which remnants were specifically identified, their elevations appear as strong predictors of CHD (Steiner et al., 1987; Tornvall et al., 1993; Hodis et al., 1994; Thompson, 1998; Takeichi et al., 1999; Karpe et al., 2001).

2.2.5. High-density lipoprotein cholesterol and cardiovascular disease

HDL cholesterol is one of the 5 major groups of lipoproteins cholesterol, which enable lipids like cholesterol and TG to be transported within the water based blood stream. In healthy persons, about thirty percent of blood cholesterol is carried by HDL cholesterol. A high level of HDL cholesterol seems to protect against CVD and low HDL cholesterol levels increase the risk for heart disease. When measuring cholesterol, some contained in HDL particles is considered as protection to the body's cardiovascular health, in contrast to "bad" LDL cholesterol.
Strong epidemiological evidence links low levels of serum HDL cholesterol to increased CHD morbidity and mortality (Abbott et al., 1988; Gordon et al., 1989; Wilson et al., 1998). High HDL cholesterol levels conversely convey reduced risk. Various epidemiological data taken as a whole suggest that a 1 percent decrease in HDL cholesterol is associated with a 2–3 percent increase in CHD risk (Gordon et al., 1989). Epidemiological studies consistently show low HDL cholesterol to be an independent risk factor for CHD. Its independent association holds after correction for other risk variables in multivariate analysis.

In fact, in prospective studies (Wilson et al., 1980; Assmann et al., 1996), HDL cholesterol usually proves to be the lipid risk factor most highly correlated with CHD risk. Adult Treatment Panel II (ATP II) specified low HDL cholesterol (<35 mg/dL) as one of several major risk factors used to modify the therapeutic goal for LDL cholesterol. The definition of low-HDL cholesterol was set to be the same for both men and women because of the view that a given level of HDL cholesterol would impart the same risk for men and women.

2.3. Nontraditional risk markers

The epidemiological and basic science search for better understanding of the etiology of CVD has produced numerous serum markers as candidates for representing “nontraditional” risk. Several are part of the progression of inflammation - a process, now understood to be central to atherosclerotic disease (Libby et al., 2002). Candidates have included homocysteine, coagulation markers such as plasminogen activator inhibitor-1 (PAI-1), fibrinogen, D-dimer and thrombin/antithrombin III complex; and various inflammatory markers such as CRP, interleukin (IL), serum amyloid A (SAA), MMP and adhesion molecule. However, many of these markers show promise, most are not used in routine clinical practice and the predictive power of many has not been confirmed.

2.3.1. High-sensitivity C-reactive protein and cardiovascular disease

Prospective epidemiologic studies with follow-up periods ranging from 3 to 20 years have found that the measurement hsCRP alone is a strong predictor of MI or CHD mortality (Ridker et al., 2002; Rifai et al., 2002; Witherell et al., 2003) stroke (Ridker et al., 2002; Cao et al., 2003) peripheral vascular diseases (Ridker et al., 2001) congestive heart failure (CHF) (Vasan et
al., 2003) atrial fibrillation, (Aviles et al., 2003) and sudden cardiac death (Albert et al., 2002) in persons without a history of CVD.

Taken in the aggregate, epidemiologic studies indicate that participants with hsCRP levels in the top quartile of the sample distribution are 2 to 3 times more likely to have a future vascular event than are those in the bottom hsCRP quartile. In most instances, the association between hsCRP and subsequent vascular events exhibits a linear “dose-response” shape and is independent of age, diabetes, hypertension, dyslipidemia and smoking, the traditional risk factors evaluated in daily practice and included in global cardiovascular prediction algorithms such as that derived from the FHS.

Data from 2 large primary prevention cohorts (8-year follow-up), the Physicians' Health Study (PHS) and the WHS, point out that, following adjustment for traditional risk factors, for each quintile raise in hsCRP, the risk of a future cardiovascular event raises by 26% for men (Ridker et al., 1997) and 33% for women (Ridker et al., 2000). The relationship between hsCRP and CVD has been established in the United States and Europe, in the middle-aged and elderly persons and in high and usual-risk populations. The relationship is evident even in studies with follow-up periods exceeding 10 years. For example, increasing hsCRP levels at baseline were predictive of 17-year coronary mortality in the MRFIT (Kuller et al., 1996) and of unexpected cardiac death in the PHS (Albert et al., 2002).

In the Honolulu Heart Program (HHP), hsCRP was a strong predictor of MI (Sakkinen et al., 2002) and thromboembolic stroke (Curb et al., 2003) up to 20 years after first blood samples had been taken. However, the HHP is 1 of only a handful of studies of nonwhite populations; data on the predictive value of hsCRP in such populations are sparse. Much of the available data on hsCRP and occurrence CVD have been obtained from nested case-control studies, which permit assessment of RRs of disease but not of absolute risks within risk factor strata.

Although, event-free survival data from several large cohorts, including the WHS, (Ridker et al., 2002) the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), (Ridker et al., 2001) the multiethnic Atherosclerosis Risk in Communities (ARIC) study in the United States, (Ballantyne et al., 2004) and the MONitoring of trends and determinants In CArdiovascular disease (MONICA) Augsburg study in Europe, (Koeing et al.,
2004) have become available, allowing a straightforward interpretation of hsCRP levels in terms of population-based quintiles or simple cut points.

The latter approach, the levels of hsCRP less than 1, 1 to 3 and greater than 3 mg/L indicates low-, moderate- and high-risk groups, respectively, provides similar predictive utility as the previous approach and has greater clinical application. Data from the WHS also prove earlier work from the PHS (Ridker et al., 1998) and AFCAPS/TexCAPS (Ridker et al., 2001) demonstrating that hsCRP levels evidently add to the predictive value of cholesterol screening. Ridker et al., (2002) endurance data shows that for individuals with LDL cholesterol above or below the study median of 124 mg/dL and hsCRP above or below the study median of 1.52 mg/L (Ridker et al., 2002).

The prognostic value of hsCRP is also an additive to that of the hypertension (Blake et al., 2003), metabolic syndrome (Ridker et al., 2003) and several markers of subclinical atherosclerosis (Park et al., 2002). Results from a study of more than 6,400 men and women from Iceland further highlight the clinical efficacy of hsCRP as an independent predictor of risk (Danesh et al., 2004). In this 20-year research, levels of hsCRP were related with an approximate 50% increase in future vascular risk not only after adjustment for traditional risk factors included in the Framingham risk score but also after further simultaneous control for diabetes, BMI, TG and indices of pulmonary function. Furthermore, in the first 10 years of follow-up, an even higher overall risk estimate was noted. These data revealed the clinical efficacy of hsCRP in a population that is relatively hyperlipidemic in assessment to available United States cohorts (Danesh et al., 2004).

While clinical interpretation of hsCRP is best performed by using ranges of less than 1, 1 to 3 and greater than 3 mg/L, data from the WHS point out that the cardiovascular risk gradient is continuous across the full spectrum of assessable hsCRP levels. The absolute risk of CVD is extremely low for those 10% to 15% of populations with hsCRP concentrations below 0.5 mg/L and the risk continues to increase as hsCRP levels exceed 10 or even 20 mg/L (Ridker and Cook, 2004). Thus, contrary to early expectations, noticeably elevated levels of hsCRP do not appear to be false-positive readings attributable to an acute-phase response. Rather, chronic elevations of hsCRP above 10 mg/L are indicative of very high cardiovascular risk.
2.3.2. Fibrinogen and cardiovascular disease

Plasma fibrinogen is an essential constituent of the coagulation cascade, in addition to that it is a major determinant of blood viscosity and blood flow. Epidemiological studies proposed that increased levels of plasma fibrinogen are related with an increased risk of CVD, including ischaemic heart disease (IHD), stroke and other thromboembolism (Meade et al., 1986; Wilhelmsen et al., 1984). The increase levels of plasma fibrinogen may promote a prothrombotic and hypercoagulable state and may in part explain the risk of thromboembolism and stroke in conditions such as atrial fibrillation. Numerous epidemiological studies have provided possible data on plasma fibrinogen levels in relation to CVD. The risk of developing a cardiovascular event such as IHD and stroke is 1.8 to 4.1 times higher in subjects with fibrinogen levels. Preliminary evidence also proposed that reducing fibrinogen levels in patients with high levels and CHD may be beneficial (Ernst and Resch, 1993).

A meta-analysis with samples representative of the general population, noted that plasma fibrinogen was an independent cardiovascular risk factor. Plasma fibrinogen was related with "true" risk factors such as hypercholesterolemia, diabetes and hypertension also included in this meta-analysis. Conversely, even when these factors were included in the multivariate analysis, the relationship between fibrinogen and CVD remained statistically significant and this suggesting that fibrinogen is an independent CVD (Ernst and Resch, 1993).

In another meta-analysis, trying to establish the role of fibrinogen as a CVD risk factor, the overall estimate of risk of cardiovascular events in subjects with plasma fibrinogen levels in the higher tertile, was double as high as that of subjects in the lower tertile. Thus, there is strong and clear evidence from epidemiological studies that fibrinogen levels are independently related to the presence of and the subsequent progress of CVD (Maresca et al., 1999).

In the Northwick Park Heart Study (NPHS) study, out of 1511 White men with age group between 40 and 64 years, 109 consequently experienced a first major IHD event (Meade et al., 1986). Increased levels of plasma factor VII coagulant activity and fibrinogen were related with increased IHD risk. Certainly, elevations of one standard deviation in cholesterol, factor VII activity and fibrinogen were associated with increases in the risk of an occurrence of IHD
within 5 years of 43%, 62% and 84%, respectively, signifying that the association between haemostatic markers and IHD to be stronger than that for cholesterol.

In the Framingham Study, the risk of developing CVD was significantly related to plasma fibrinogen levels. Evenly in both sexes, cardiovascular and stroke risk increased progressively in relation to antecedent fibrinogen. The impact of plasma fibrinogen levels on CVD was comparable with the major risk factors, such as hypertension, adiposity, cigarette smoking and diabetes; and was still an independent predictor of CHD on multivariate analysis (Kannel et al., 1987; Kannel et al., 1992).

In the Munster Heart Study (MHS), lipid parameters, blood pressure, plasma fibrinogen and factor VIIc were measured in 2781 healthy men aged 40–65 years. 130 coronary events were observed after the 8 years of follow-up and the mean plasma fibrinogen level of the "event group" exceeded than the non-event group (Assmann et al., 1996). The Caerphilly and Speedwell collaborative heart disease studies were based on a combined cohort of 4860 middle-aged men from the general population. After 5.1 years of follow-up, 251 major IHD events occurred. Multivariate analysis showed that fibrinogen, white blood cell count and viscosity were independent risk factors for IHD (Yarnell et al., 1991).

In the European Concerted action on thrombosis and disabilities study (ECAT), plasma fibrinogen was a strong and independent risk factor for MI and sudden death, mainly in patients with pre-existing CHD, along with plasma tissue plasminogen activator antigen and von Willebrand factor antigen. In patients with CHD, the association of plasma fibrinogen levels to the occurrence of ACS was stronger than that of LDL cholesterol, tissue plasminogen activator antigen and von Willebrand factor antigen (Thompson et al., 1995).

As with other conventional risk factor, very rarely studies have unsuccessful to provide the predictable results that associating fibrinogen with CHD. In the prospective Gottingen Risk Incidence and Prevalence Study (GRIPS) based on a sample of 6002 men aged 40–60 years, initially free of CVD, plasma fibrinogen was an independent risk factor for the occurrence of acute coronary events during the initial 5 years of follow-up, however, this association was lost during the subsequent 5 years of follow-up. This may be partly recognized to the lack of
consistent recommendations for the increased plasma fibrinogen levels and choosing different cut-off points (Cremer et al., 1994; Cremer et al., 1996).

2.3.3. Tumor necrosis factor alpha and cardiovascular disease

Recent research in both animal models and humans provides convincing evidence identifying TNF-α as one of several regulators of vascular homoeostasis. NO is a free radical produced by NOS (NO synthase) in a two-step five-electron oxidation of the terminal guanidino nitrogen of L-arginine (Moncada et al., 1991).

TNF-α controls the expression and activity of NOS which exerts direct effects on the production of NO (MacNaul et al., 1993). Further studies have also shown that TNF-α considerably decreased eNOS expression in ECs (Xia et al., 2006; Goodwin et al., 2007) and consequently these contributes to nitrate stress and impair endothelial function.

Several studies suggests that TNF-α impairs endothelium-dependent and NO-mediated vasodilation in various vascular beds, for example, mouse coronary arterioles (Gao et al., 2007), rat coronary arterioles (Picchi et al., 2006), cat carotid arteries (Aoki et al., 1989) and bovine small coronary arteries (Ahmad et al., 2002).

Picchi et al. (2006) suggested that endothelial dysfunction in pre-diabetic metabolic syndrome may be as a result of the effects of TNF-α and the subsequent production of O$_2^{•-}$. The contribution of TNF-α in ischaemia/reperfusion injury in TNF 1.6 mice, which over express TNF-α in cardiac tissue. Myocardial ischaemia/reperfusion increase in the expression of TNF-α, which induced activation of xanthine oxidase and the production of O$_2^{•-}$, leading to coronary endothelial dysfunction (Zhang et al., 2006).

Gao et al. (2007) demonstrated that advanced glycation end-product/receptor for advanced glycation end-product and NF-κB signalling play a pivotal role in elevating circulating and/or local vascular TNF-α production. The elevated TNF-α expression induces the production of ROS, leading to endothelial dysfunction in patients with type 2 diabetes. Endothelial dysfunction related with TNF-α in pathophysiological condition is linked to excess production of ROS and a decrease in NO bioavailability, which appears to affect several aspects of CVD.
2.3.4. Interleukin-6 and cardiovascular disease

IL-6 is an IL that acts as pro-inflammatory cytokine as well as an anti-inflammatory myokine. The Women’s Health and Aging study (WHAS) demonstrated that women with CVD, those with elevated plasma IL-6 levels had a more than fourfold risk of death compared with women in the lowest tertile, but the study did not find this relationship among those without CVD (Volpato et al., 2001).

In contrast, Tuomisto et al. (2006) study showed that CRP and TNF-α, but not IL-6 were significant independent predictors of total mortality among men. However, Scharnagl et al. (2010) found IL-6 to be more strongly related with all-cause and cardiovascular mortality than hsCRP. Other recent studies have revealed that increased levels of serum IL-6 provide valuable information for the risk evaluation of long-term cardiovascular and all-cause mortality (Fan et al., 2011; Rao et al., 2005).

A recent study established that plasma IL-6 level predicts both short- and long-term mortality in patients with acute heart failure (Pudil et al., 2010). In two other prospective studies, long-term serum IL-6 levels were related with CHD (Danesh et al., 2008). Haugen et al. (2008) showed that an increased IL-6 concentration predicts mortality in elderly heart failure patients. Panichi et al. (2004) reported plasma IL-6 to be a stronger predictor of total and cardiovascular mortality than CRP in patient’s undergone haemodialysis.

Recently, Dongfang et al. (2013) confirmed these reports that the positive relations between circulating IL-6 concentration and subsequent risk of mortality, resulting that serum IL-6 is a stronger predictor of total and cardiovascular mortality than CRP, which supports the probable role of inflammation in the progression and prognosis of CHD.

The possible mechanisms by which circulating IL-6 contributes to the pathogenesis of CHD are, first, serum IL-6 is the major stimulator of hepatic acute-phase response, which is related with increased blood viscosity and elevated level of platelet number and its activity. Second, the paracrine and autocrine activation of monocytes by IL-6 in the vessel wall leads to the deposition of fibrinogen (Van Der Poll et al., 1994). Third, IL-6 reduces the activity of lipoprotein lipase, thus increasing the uptake of lipids by macrophages (Hardardottir et al.,
Fourth, the circulating IL-6 also enhances the hypothalamic-pituitary-adrenal axis, the activation of which is related with hypertension, obesity and insulin resistance (Mastorakos et al., 1993).

**2.3.5. Lipoprotein (a) and cardiovascular disease**

Lp(a) is an LDL-like molecule consisting of an apolipoprotein B-100 [apo(B-100)] particle attached by a disulphide bridge to apo A-I. Numerous observations have pointed out that Lp(a) levels may be a risk factor for CVD. Elevated serum Lp(a) is an independent predictor of CHD and MI (Schaefer et al., 1994; Bostom et al., 1994).

Motta et al. (2001) examined the transient increased serum levels of this lipoprotein during acute myocardial infarction (AMI). The positive association between mean values of Lp(a) on day 1 and 7 and the size of the necrotic area suggested an atherogenic and prothrombotic role of Lp(a). Additionally, elevated Lp(a) values were associated to greater tissue damage and it has been suggested that periodical determination of Lp(a) values in subjects with coronary disease may be useful in order to predict further acute vascular events.

Elevated serum Lp(a) level might be a high-risk factor for clinical coronary stenosis progression and restenosis. Increased Lp(a) concentration is a significant predictor of long-term adverse outcome in AMI patients treated by primary percutaneous transluminal coronary angioplasty (Igarashi et al., 2003). Serum Lp(a) levels ≥ 25 mg/dL are identified in 67% of patients with rapid development of CHD but in only 33% of patients without development of CHD (Terres et al., 1995).

Tamura et al. (1995) evaluated the association between serum Lp(a) level and angiographically assessed CHD progression without new MI, reporting a significant relationship. A meta-analysis confirmed that Lp(a) levels can be measured as a risk factor for CVD (Craig et al., 1998; Danesh et al., 2000). Sandholzer et al. (1992) reported that in premature CHD patients, the alleles at apo A-I locus determine risk for CHD through their effect on plasma Lp(a) level. The results of this study recommended that Lp(a) can be considered a main genetic risk factor for CHD.
The Copenhagen City Heart Study (CCHS) found that very high Lp(a) levels > 95th percentile predict a 3- to 4-fold increase in risk of MI and absolute 10-year risks of 20% and 35% in high-risk women and men (Kamstrup et al., 2008). For the first time CCHS provided absolute 10-year risk estimates in the general population for MI and ischemic heart disease as a function of Lp(a) levels stratified for other risk factors, allowing clinicians to utilize extreme Lp(a) levels in risk assessment of individual patients (American Association for Clinical Chemistry, 2009).

Lp(a) screening is not necessary for primary prevention and evaluation of cardiovascular risk at present, but that Lp(a) measurements can be valuable in patients with a strong family history of CVD or if risk of CVD is considered intermediate on the basis of conventional risk factors (Milionis et al., 2000). The European Atherosclerosis Society Consensus Panel have recommended the screening for elevated Lp(a) in those at intermediate or high CVD/CHD risk, a desirable level < 50 mg/dL as a function of global cardiovascular risk (Nordestgaard et al., 2010).

2.3.6. Apolipoproteins and cardiovascular disease

Apolipoproteins are the protein components of plasma lipoproteins and numerous types of apolipoproteins have been recognized. The most important apolipoprotein constituent of LDL cholesterol is apo B, is the chief protein component constituent of the atherogenic VLDL cholesterol, of IDL and of LDL particles, each particle including one apo B molecule. Therefore, plasma apo B levels reflect the total numbers of atherogenic particles. Apo B is necessary for the binding of LDL particles to the LDL receptor for cellular uptake and degradation of LDL particles.

Apo A-I is the most important apolipoprotein constituent of the antiatherogenic HDL cholesterol. Concentrations of apo A-I are strongly related with those of HDL cholesterol. Apo A-I is critically concerned in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport, either directly or indirectly via LDL cholesterol to the liver. It is remarkable that apo B, apo A-I and the apo B to apo A-I ratio reflect the status of the major atherogenic and anti-atherogenic pathways of lipoprotein metabolism. Consequently, high apo B, high apo B to apo A-I ratio and low apo A-I levels in plasma indicate a high risk for CVD and
vice versa (Chan et al., 2004). Conditions that selectively elevate LDL cholesterol will increase apo B levels and conditions which lower HDL cholesterol will lower apo A-I (Eckel et al., 2005).

Several epidemiological studies that have shown that apolipoproteins are better predictors of cardiovascular risk than traditionally measured lipids, particularly LDL cholesterol and HDL cholesterol (Shai et al., 2004; Meisinger et al., 2005). Quebec Cardiovascular Study was the first prospective study to reveal strongly that apo B was greater to cholesterol indices in predicting CHD risk (Lamarche et al., 1996). In a sample of 2,155 Canadian men followed for a period of 5 years for clinical signs of IHD, plasma apo B concentrations showed a strong relationship with onset of IHD independent of TG, HDL cholesterol and TC to HDL cholesterol ratio. St-Pierre et al., (2005) reported that elevated plasma apo B levels remained an independent risk factor for IHD. This study also found that the association of high levels of apo B and an increased risk of IHD.

High apo B and low apo A-I levels were noteworthy predictors of recurrent coronary events, which is independent for other lipid variables such as TC, LDL cholesterol and TG. In Apolipoprotein-related MOrtality RISk (AMORIS), a total of 1,75,553 participants from Sweden were included and followed for an average of 5.5 years. The association between fatal MI and apolipoproteins and other lipid measures were evaluated. In multivariate analyses, apo B, apo A-I and apo B to apo A-I ratio were all more significant predictors of MI in both sexes. Receiver operating characteristics analysis also supported that apo B had higher sensitivity and specificity than LDL cholesterol as a predictor, particularly in those with normal/low LDL cholesterol level (Walldius et al., 2001).

In Second Northwick Park Heart Study (NPHS II), a total of 2,508 healthy middle-aged United Kingdom men were included to examine the relative values of apo B and other lipid variables in predicting CHD risk over 6 years of follow-up (Talmud et al., 2002). In univariate analyses, the RRs for LDL cholesterol, apo B, apo A-I and apo B to apo A-I ratio were 2.67, 2.90, 0.52 and 3.58, respectively.

Evidently, the apo B to apo A-I ratio conferred the highest RR of CVD. INTERHEART Study assessed that the relative importance of risk factors for CHD in 15,152 cases and 14,820 controls recruited from 52 countries worldwide (Yusuf et al., 2004). The ratio of apo B to apo A-I
was the strongest risk factor in predicting MI, followed by current smoking, psychosocial factors, diabetes, hypertension and abdominal obesity. Waldius et al., (2004) confirmed that the apo B to apo A-I ratio is greater to any of the cholesterol ratios as a summary index of the lipoprotein-related risk of vascular disease. In both the INTERHEART and AMORIS studies, the influences of apo A-I and apo B were independent and equivalent.

Because of the complexity and inter-relatedness no single biomarker can capture all of the important risk information. Markers can also be combined to increase the specificity of the definition of CVD.

2.4. Lipid-modulating agents

Lipid-modulating medications reported to have anti-inflammatory properties, which includes HMG-CoA reductase inhibitors (Statins), niacin and fibrates. Of these, the findings for statins are by far the most robust.

2.5. Statins

Statins are widely used as lipid-lowering drugs in patients with CVD. For reasons of its cost-effectiveness, statin therapy for primary prevention is restricted to patients with overt hyperlipidemia. Though, the clinical and pathological effects of statins cannot be recognized to the lowering of circulating LDL cholesterol alone. Among their other effects, statins may also have anti-inflammatory properties (Lyn and Uzick, 2001). Numerous studies have confirmed the hsCRP-lowering capacity of statins, independent of changes in lipid profiles (Ridker et al., 2001). This may also have implications for the use of statins in primary prevention in individuals without hyperlipidemia.

2.6. Beneficial and Pleiotropic effects of Statins (particularly Atorvastatin)

2.6.1. Statins and cholesterol

Cholesterol is an important constituent of cell membranes and is the immediate precursor of bile acids and steroid hormones (Goldstein and Brown, 1990). Conversely, in excessive amounts, cholesterol becomes a significant risk factor for CVD, as demonstrated in clinical trials from the FHS (Gordon and Kannel, 1971) and the MRFIT (Multiple risk factor
intervention trial, 1982). While dietary cholesterol can contribute to changes in serum cholesterol levels, two thirds of the body’s cholesterol is synthesized by liver.

Hence, inhibition of hepatic cholesterol biosynthesis has emerged as the target of option for reducing serum cholesterol levels (Panel NCEPE, 2002). HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis in the liver (Goldstein and Brown, 1990), which catalyzes the conversion of mevalonic acid from HMG-CoA (Rodwell et al., 1976). Inhibitors of HMG-CoA reductase were originally recognized as secondary metabolites of fungi (Alberts, 1988).

Endo et al. (1976) isolated the first natural inhibitors of HMG-CoA reductase was mevastatin was isolated from Penicillium citrinum. Consequently, a more active fungal metabolite, lovastatin or mevinolin, was isolated from Aspergillus terreus (Alberts, 1990). Later, several other new statins have become commercially available, both chemically modified and natural statins, including simvastatin, cerivastatin, pravastatin, fluvastatin, atorvastatin and most recently, pitavastatin and rosuvastatin (Illingworth and Tobert, 2001).

Certainly, statins have emerged as one of the most effective class of agents for reducing serum cholesterol levels. Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme’s active site and block the substrate-product transition state of the enzyme (Istvan and Deisenhofer, 2001). Thus, all statins share an HMG-like moiety and inhibit the reductase by similar mechanism. There are slight differences in the modes of binding between the various statins, with the synthetic compounds atorvastatin and rosuvastatin found to have the greatest number of bonding interactions with HMG-CoA reductase (Istvan and Deisenhofer, 2001).

Atorvastatin is one of the most usually prescribed statin in the world. Guidelines published in 2011 by the European Atherosclerosis Society and the European Society of Cardiology recommend a goal of either LDL cholesterol <70 mg/dl or ≥ 50% reduction in LDL cholesterol for patients at very high cardiovascular risk. As statin dose increased, higher percentages of patients achieved LDL cholesterol <70 mg/dl or ≥ 50% LDL cholesterol reduction. A greater percentage achieved this goal with rosuvastatin 10-40 mg than with equal or double milligram doses of atorvastatin or simvastatin. These data from VOYAGER highlight
the importance of an effective statin at an appropriate dose to achieve treatment goals for LDL cholesterol in patients with very high cardiovascular risk (Karlson et al., 2013).

In Multiple-Intervention-in-type-2-Diabetes.ITaly (MIND.IT) study suggested that, the change in LDL cholesterol appears to be more related to an increased number of treated patients and a decreased treatment withdrawal than to a true treat-to-target approach (Ardigo et al., 2012; Oh et al., 2012). Conventional doses of pitavastatin and atorvastatin effectively and safely reduce elevated hepatic enzyme concentrations (Han et al., 2012). SNP rs8192870, located in the first intron of the CYP7A1 gene, may be associated with the LDL level lowering response to atorvastatin (Jiang et al., 2012).

Atorvastatin treatment increased cholesterol absorption by 52.3% in those with the lowest baseline campesterol levels, which attenuated the effect of LDL cholesterol reduction. Especially those with the highest lathosterol but the lowest campesterol levels at baseline had significantly less LDL cholesterol reduction than those with the same baseline lathosterol levels but the highest campesterol levels. These results suggest that combined patterns of cholesterol synthesis/absorption markers, relatively than each single marker, are potential predictors of the LDL cholesterol lowering effects of atorvastatin in high-risk CHD patients (Qi et al., 2013).

Atorvastatin lowered P wave dispersion significantly and this finding may be important in the prevention of atrial fibrillation in hyperlipidemic patients (Ayca et al., 2013). Treatment responses to atorvastatin and ezetimibe/simvastatin in at-risk patients with the metabolic syndrome were related to age (≥ 65 years), abdominal obesity and lower baseline hsCRP (Robinson et al., 2013). Recently, combination treatment with atorvastatin and ezetimibe had relatively better lipid-lowering and anti-inflammatory efficacy than atorvastatin monotherapy (Padhy et al., 2013). Pravastatin and atorvastatin of 10 mg per day each increased HDL cholesterol by almost the same extent (Sasaki et al., 2013).

Serum high mobility group box 1 protein (HMGB1) has been identified as a novel pro-inflammatory cytokine in CHD. HMGB1 levels are increased in patients with hyperlipidemia which could be reduced by atorvastatin (Jin et al, 2012). Rodrigues et al. (2013) study highlighted the current knowledge that carrying APOB rs693 is an independent risk factor for dyslipidemia and higher LDL cholesterol levels.
Furthermore, it has been found that a variant of CD36 was associated with dyslipidemia as a risk (rs1984112) factor. Atorvastatin response could be predicted by LIPC -514C>T SNP and physical activity. These data evidences the contribution of genetic markers and their interaction with environmental factor in the variability of statin response.

2.6.2. Statins and endothelial function

The vascular endothelium serves as a most important autocrine and paracrine organ that controls vascular wall contractile state and the composition of the cellular component. Increased level of cholesterol impairs the endothelial function. Endothelial dysfunction is one of the initial manifestations of atherosclerosis, occurring even in the lack of angiographic evidence of disease (Liao et al., 1991; Libby, 1995). The main characteristic of endothelial dysfunction is the diminished synthesis, release and activity of endothelial derived NO and it has been shown to inhibit several components of the atherogenic process. Endothelium-derived NO mediates vascular relaxation (Ignarro et al., 1987), vascular smooth muscle proliferation (Garg and Hassid, 1989), inhibits platelet aggregation (Radomski et al., 1992) and endothelial-leukocyte interactions (Gauthier et al., 1995). Inactivation of NO by superoxide anion (O2\textsuperscript{−}) confines the bioavailability of NO and leads to nitrate tolerance, hypertension and vasoconstriction (Harrison, 1997).

Statins could restore endothelial function, relatively, by lowering serum cholesterol levels. Conversely, in a few studies with statins, restoring of endothelial function occurs before significant reduction in serum cholesterol levels (Treasure et al., 1995; O’ Driscoll et al., 1997), signifying that there are some more beneficial effects on endothelial function other than that of cholesterol reduction. Certainly, statins increase endothelial NO formation by stimulating and upregulating eNOS (Kureishi et al., 2000). Statins have been shown to restore the activity of eNOS in the presence of hypoxia (Laufs et al., 1997) and ox-LDL cholesterol (Laufs et al., 1998). Additionally, statins increase the expression of tissue-type tissue plasminogen activator antigen (Essig et al., 1998) and inhibit the expression of endothelin-1 (Hernandez-Perera et al., 1998).

Glucose loading blunted endothelial function. Combined treatment with metformin and atorvastatin for 6 weeks partly prevented the glucose-induced impairment of endothelium-dependent dilation in patients received treatment, with a significant difference compared with
monotherapy with metformin (Tousoulis et al., 2010). Atherogenic risk in subjects with metabolic syndrome is partly mediated by increased oxidative stress and subsequent endothelial dysfunction. Clinical trials have confirmed differences in outcomes between subjects receiving atorvastatin (lipophilic) compared with pravastatin (hydrophilic). Atorvastatin therapy was associated with significant reduction in plasma thiobarbituric acid reactive substances and lipid hydroperoxides levels, which was not noted in subjects treated with pravastatin (Murrow et al., 2012).

Atorvastatin treatment simultaneously enhanced expression of endothelial nitric oxide (eNO) synthase and yielded of NO and cyclic guanosine monophosphate. Zhang et al. (2010) study proved the hypothesis and may be pertinent to the complex mechanism of action of statins explaining their long-term beneficial effects in maintaining the morphological and functional integrity of vascular EC.

Arterial stiffness indicated endothelial dysfunction. There was reduction in reflection index with treatment of N-acetylcysteine and atorvastatin signifying the improvement in endothelial dysfunction. There was decrease in hsCRP and malondialdehyde after treatment with N-acetylcysteine suggesting improvement in endothelial dysfunction. There was reduction in hsCRP after treatment with atorvastatin, indicating the improvement in endothelial function and this is associated with decreased incidence of cardiovascular and cerebrovascular accidents (Kudarvalli, 2011).

Jaumdally et al. (2011) hypothesized that abnormal endothelial (marked by von Willebrand factor), angiogenesis (vascular endothelial growth factor, angiopoietins 1 and 2) and platelet function (soluble P selectin, soluble CD40L) improve with atorvastatin treatment in diabetics. Treatment increased angiopoietin-2 in all groups regardless of diabetes. In those free of diabetes, angiopoietin-2 increased 3-fold, while in diabetes, it increased 2-fold. It has been suggested that an additional effect of statins is to increase levels of growth factor angiopoietin-2 in the direction of normality.

Recently, a randomized, double-blind study compared the short-term effects of rosvastatin and atorvastatin on serum lipids and markers of inflammation and endothelial function in patients with stable atherosclerosis. Both statins significantly reduced Rho-
associated coiled-coil containing protein kinase (ROCK) activity and inhibition was significantly greater with rosvastatin. There was no association between ROCK activity and LDL cholesterol level in either group. There was a significant relationship between ROCK activity and flow-mediated dilation for both statins. Short-term treatment with either rosvastatin or atorvastatin inhibits ROCK activity independent of decrease in cholesterol level and improves endothelium dysfunction in patients with atherosclerosis (Liu et al., 2011).

Small GTPases (guanosine triphosphate, GTP) are concerned in many critical cellular processes, including inflammation and proliferation. GTP loading and isoprenylation are two significant post-translational modifications of small GTPases and are critical for its normal function. Atorvastatin treatment of human umbilical vein EC produced a time-dependent increase in GTP loading of all Rho GTPases and induced the translocation of small Rho GTPases from the cellular membrane to the cytosol. Atorvastatin significantly attenuated thrombin-induced human umbilical vein EC permeability, elevated VE-cadherin targeting to cell junctions and preserved junction integrity. Atorvastatin treatment increased GTP loading and inhibited isoprenylation of small GTPases, accompanied by decreased inflammatory response and preserved cellular junction integrity (Xiao et al., 2013).

2.6.3. Statins and anti-oxidant effects

Antioxidant effects of statins are another potential mechanism for improving endothelial function. Statins enhance endothelium dependent relaxation by inhibiting the production of ROS, such as superoxide and hydroxy radicals, from aortas of cholesterol-fed rabbits (Rikitake et al., 2001). Although lipid lowering by itself can lower vascular oxidative stress (Cai and Harrison, 2000), some of these antioxidant effects of statins appear to be cholesterol-independent. Statins attenuate angiotensin II-induced free radical production in VSMCs by inhibiting Rac1-mediated NADH oxidase activity and downregulating AT1-R expression (Wassmann et al., 2001). Since NO is scavenged by ROS, these conclusion indicate that the antioxidant properties of statins may also contribute to their ability to improve endothelial function (Harrison, 1997).

Atorvastatin treatment enhanced the lipid profile, lipid oxidation, oxidative and antioxidative status markers as well as the activity of PON1 towards paraoxon. These beneficial
effects may be recognized to the antioxidant potential of statins and the increase in PON1 activity. The increase in PON1 activity was improved by the PON1 T-107C polymorphism (Nagila et al., 2009). Carnevale et al. (2010) study provides the evidence that in patients higher adiponectin serum levels are associated with gp91(phox) down-regulation. Adiponectin-mediated gp91(phox) reduction could be one of the mechanisms involved in atorvastatin’s antioxidant effect.

Diabetes is associated with abnormalities in lipid profile and increased oxidative stress. Both atorvastatin and rosuvastatin may be helpful in reducing increased oxidative stress in diabetic patients with hyperlipidemia (Koksal et al., 2011). Atorvastatin exerted antioxidative and antiperoxidative effects in patients with CHD and dyslipidemia. It normalized parameters of lipid peroxidation and antioxidant protection thus improving the clinical course of CHD (Bondar et al., 2012). Pignatelli et al. (2012) study provides the first evidence that atorvastatin acutely and simultaneously decreases oxidative stress and platelet activation by directly inhibiting platelet Nox2 and eventually platelet isoprostanes and thromboxane A(2). These results provide a basis for the use of statins to prevent or modulate coronary thrombosis.

Recently, Sozer et al. (2013) findings concluded that the atorvastatin had HDL-related antioxidant activity as well as lipid-lowering properties in L-NAME treated rats. Atorvastatin also had a clear inhibitory effect on the development of bladder cancer, possibly due to its antioxidant, anti-inflammatory and anti-proliferative properties (Parada et al., 2012). Atorvastatin has remarkable antiatherosclerotic effects, part of which appears to be due to the antioxidant features of the parent drug and/or its metabolites, favoring inhibition of LDL oxidation (Sezer et al., 2011). Hypoxia induces the formation of ROS, myocardin expression and cardiomyocyte hypertrophy can be prevented by atorvastatin by suppressing ROS and myocardin expression (Chiu et al., 2012).

2.6.4. Statins and endothelial progenitor cells

Endothelial progenitor cells (EPCs) are a subtype of hematopoietic stem cells, which contribute to the restore of injured endothelium. EPCs enhance ischemia-induced neovascularization (Murohara et al., 2000), accelerate re-endothelialization following carotid balloon injury (Walter et al., 2002; Werner et al., 2002) and enhance postischemic cardiac
function (Kawamoto et al., 2001). Landmesser et al. (2004) findings suggest that increased eNO availability is required for statin-induced improvement of endothelial progenitor cell mobilization, neovascularization, left ventricular dysfunction, myocardial neovascularization and survival after MI. eNO bioavailability after MI possibly represents an important therapeutic target in heart failure after MI and mediates beneficial effects of statin treatment after MI.

In patients with stable CHD, administration of statins for four weeks improved the number of circulating EPCs and enhanced functional capacity in stable CHD patients (Vasa, et al., 2001). Minami et al. (2009) study demonstrates that lipid lowering therapy with atorvastatin increases EPCs numbers and decreases microRNA-221/222 levels in CHD patients, probably contributing to the favorable effects of lipid lowering therapy with atorvastatin in this disorder. Atorvastatin pretreatment significantly increases the amount of EPCs after cardiopulmonary bypass surgery, by a mechanism independent of plasma levels of cytokines and cholesterol (Spadaccio et al., 2010).

Atorvastatin inhibited homocysteine-induced dysfunction and apoptosis in EPCs, which may be related to its effects on suppressing oxidative stress through up-regulating Akt/eNOS and down-regulating the p38MAPK/caspase-3 signaling pathway (Bao et al., 2010). Atorvastatin also antagonized homocysteine-induced activation of NADPH oxidase and overexpression of Nox4 mRNA and p-p38MAPK protein. Similar effects happen with EPCs transfected with Nox4 siRNA. These findings established that atorvastatin may inhibit homocysteine-induced NADPH oxidase activation, accumulation of ROS and EPCs apoptosis through Nox4/p38MAPK dependent mechanisms and this may contribute to atorvastatin-induced beneficial effects on EPCs function (Bao et al., 2010).

At higher concentrations, atorvastatin therapy in pre-percutaneous coronary interventions improves EPCs number and cultured angiogenic cells number and function in humans which may in part explain the benefit in clinical outcomes seen in patients undergoing coronary interventions (Hibbert et al., 2011). Use of 40 mg of atorvastatin may decrease the levels of circulating endothelial-derived microparticles and increase the number of circulating EPCs in ischemic cardiomyopathy patients when compare with 10 mg of atorvastatin and the effect may be independent of the decrease of lipids, oxLDL and hsCRP (Huang et al., 2012).
2.6.5. Statins and smooth muscle proliferation

VSMC are essential for maintaining vasculature homeostasis and function. Several studies have shown that statins attenuate vascular proliferative disease, for example transplant-associated arteriosclerosis (Braun-Dullaeus et al., 1998). Chronic treatment with atorvastatin directly decreases mitogen-induced nuclear Ca\(^{2+}\) mobilization (Wamhoff et al., 2002). In aortic SMC atorvastatin and mevastatin significantly inhibited bFGF-induced mRNA expression of endothelin ET(A) and ET(B) receptors. Furthermore, the specific antagonists of ET(A) and ET(B) receptors significantly inhibited bFGF-induced SMC proliferation. It has been suggested that endothelin receptors and the mevalonate pathway were involved in bFGF-induced SMC proliferation (Xu et al. 2002).

Bruemmer et al. (2003) findings demonstrate that minichromosome maintenance (MCM) proteins play an essential role during the proliferation of vascular SMC and may provide a novel therapeutic target for proliferative vascular diseases. Inhibition of MCM6 and MCM7 expression through blocking of E2F function may contribute importantly to the inhibition of vascular SMC DNA synthesis by atorvastatin. Chandrasekar et al. (2006) results indicate that the proatherogenic cytokine such as, interleukin-18 (IL-18) induces human coronary artery SMC migration in an MMP9-dependent manner. Atorvastatin suppress IL-18-mediated aortic SMC migration and has therapeutic potential for attenuating the progression of atherosclerosis and restenosis.

Erythropoietin directly stimulates the proliferation of vascular SMC and this is believed to be one of the mechanisms of vascular access failure of hemodialysis patients. Statins inhibited erythropoietin-induced proliferation in rat VSMCs at least partly through their inhibition of HMG-CoA reductase activity (Kaneda et al., 2010). Lipophilic statins exert direct effects on distal human pulmonary artery SMC and are likely to involve inhibition of Rho GTPase signaling (Ali et al., 2011). Atorvastatin inhibition of periostin expression induced by TGF-β1 in VSMCs may be exerted by inhibition of the production of MVA and other isoprene compounds and by blocking the Rho/Rho kinase signaling pathway (Li et al., 2012).

Leptin contributes to the pathogenesis of atherosclerosis. Angiotensin II increases leptin synthesis in cultured adipocytes. Statin decreases the leptin expression in adipocytes and
human coronary artery ECs. Angiotensin II induces leptin expression in human VSMCs and atorvastatin can suppress the leptin expression induced by angiotensin II. The inhibitory effect of atorvastatin on angiotensin II-induced leptin expression was mediated by Rac, ROS and JNK pathways (Shyu et al., 2012).

Recently, it has been suggested that statins may also modulate VSMC activation by their influence on the rennin-angiotensin system. In VSMC culture Ang-(1-7) was identified as a major product of Ang I metabolism. In this setting TNF-α caused a decrease in the conversion of Ang I to Ang-(1-7). This consequence was accompanied by a decrease of mRNA expression of neutral endopeptidase and angiotensin converting enzyme 2 and increase of mRNA of angiotensin converting enzyme (Suski et al., 2013).

Interestingly, atorvastatin attenuated the effects of TNF-α on Ang-(1-7) production as well as reversed the influence of TNF-α on angiotensin converting enzyme and angiotensin converting enzyme 2 expression. Enhancement by atorvastatin of the ACE2/Ang-(1-7) axis in VSMCs could represent a new and beneficial mechanism on cardiovascular action of this widely used drug (Suski et al., 2013).

2.6.6. Statins and platelet function

Platelets play a vital role in the development of ACS (Fitzgerald et al., 1986). The levels of circulating platelets are associated with mural thrombus formation at the site of plaque rupture and vascular injury (Lacoste et al., 1995; Willerson et al., 1989). Hypercholesterolemia is associated with increases in platelet reactivity (Opper et al., 1995). These abnormalities are associated to increases in the cholesterol/phospholipid ratio in platelets. Additional potential mechanisms include increases in thromboxane A2 (TXA2) biosynthesis (Notarbartolo et al., 1995), platelet a2-adrenergic receptor density (Baldassarre et al., 1997) and platelet cytosolic calcium (Le Quan Sang et al., 1995).

Atorvastatin, had a marked reduction effect on platelet aggregation (Tekten et al., 2004). Haemorrheological parameters and endothelial function are known to be altered in vascular diseases, including stroke. Atorvastatin short-term and low-dose therapy can improve haemorrheological parameters and platelet aggregation endothelial dysfunction (Szapary et al.,
Combining clopidogrel with atorvastatin in the healthy individuals led to a reduction in ADP-induced platelet p-selectin exposure. Clopidogrel reduced platelet reactivity in patients with CHD simultaneous atorvastatin medication. Pretreatment with atorvastatin reduces platelet reactivity before administration of clopidogrel. There was no drug interaction noted, conversely, platelet inhibitory effects were observed during the treatment with clopidogrel and atorvastatin (Piorkowski et al., 2004).

Atorvastatin significantly reduced platelet TXA(2) synthesis and collagen-induced aggregation. Atorvastatin combined with aspirin early in the onset of the acute event significantly reduced persistent TXA(2) and TXA(2)-dependent aspirin resistance. This may be contribute to the clinical benefit of atorvastatin in patients with MI (Santos et al., 2009).

Among percutaneous coronary intervention treated patients with high on-treatment platelet reactivity during co-administration of atorvastatin with clopidogrel, switching to a non-CYP3A4-metabolized statin considerably decrease platelet reactivity and the prevalence of high on-treatment platelet reactivity. This switching effect appears similar irrespective of the type of non-CYP3A4-metabolized statin (Park et al., 2012).

Therapy with statins beneficially modifies ADP-induced platelet aggregation in patients with hyperlipidemia and does not affect spontaneous or ADP-induced platelet adhesion to fibrinogen and platelet aggregation induced by collagen or ristocetin (Sikora et al., 2013). Moscardo et al. (2013) reported the direct downregulation by atorvastatin and simvastatin of platelet cPLA2 activity through effects on calcium, MAPK and decrease TXA2 synthesis, which induced by collagen. These mechanisms might contribute to their favorable effects, even in patients treated with aspirin.

### 2.6.7. Statins and plaque stability

Plaque rupture is a main reason of ACS (Libby, 1995). The atherosclerotic lesion contains highly thrombogenic substances in the lipid core that are separated from the bloodstream by a fibrous cap (Fernandez-Ortiz et al., 1994). Ulceration of the fibrous cap ultimately leads to plaque rupture and ensuing thrombosis (Fuster et al., 1990). Collagen is the major constituent of fibrous caps, since macrophages are capable of degrading the collagen-containing fibrous cap,
they play an significant role in the progress and subsequent stability of atherosclerotic plaques (Shah et al., 1995).

Certainly, degradation of the plaque matrix appears to be most active in macrophage-rich regions (Fuster, 1995). Secretion of MMPs by activated macrophages may weaken the fibrous cap, mostly at the “vulnerable” shoulder region where the fibrous cap joins the arterial wall (Henney et al., 1991). This weakened fibrous caps lead to plaque instability, rupture and ensuing thrombosis (Libby, 1995; Davies, 1995). Intensive lipid lowering by statins can enhance plaque stability by reducing plaque size or by modifying the physiochemical properties of the lipid core (Koh, 2000; Fukumoto et al., 2001).

The plaque-stabilizing properties of statins are mediated through a combined reduction in lipids, macrophages and MMPs (Crisby et al., 2001). These effects of statins may reduce the incidence of ACS by lessening the propensity for plaque to rupture and may explain the rapid time course of event reduction in patients at high risk for recurrent coronary ischemia in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) (Schwartz et al., 2001) and Pravastatin or Atorvastatin Evaluation and Infection trials (PROVE-IT) (Cannon et al., 2004).

It is hoped that a rising awareness of the benefits of early statin therapy to stabilize culprit lesions in ACS will lead to an increase in the proportion of coronary patients who will receive this beneficial therapy (Waters, 2001). Shimojima et al. (2012) results confirmed that plaque composition and volume might be changed within 3 weeks following intensive lipid lowering therapy. This may explain acute effects of statins in treatment of ACS.

Cyclooxygenase (COX)-2 expressions is increased in inflammation and angiogenesis and also in atherosclerotic plaques, where it co-localizes with MMPs involved in weakening of the fibrous cap. The regulation of COX-2 and MMP-9 expression advocates the involvement of a Rho-dependent pathway. In the human vascular endothelium, statins (simvastatin and atorvastatin) reduce COX-2 and MMP-9 expression and activity. Through this mechanism, statins concern an anti-angiogenic effect possibly contributing to the cholesterol-lowering-unrelated protective effects of statins against plaque inflammatory angiogenesis and rupture (Massaro et al., 2010).
2.6.8. Statins and vascular inflammation

Atherosclerosis is a complex inflammatory process that is illustrated by the presence of monocytes or macrophages and T lymphocytes in the atheroma (Ross, 1993; Ross, 1999). These macrophages and T lymphocytes secreted inflammatory cytokines, which can modify endothelial function, VSMC proliferation, collagen degradation and thrombosis (Libby, 1995). An early step in atherogenesis involves monocyte adhesion to the endothelium and penetration into the subendothelial space (Ross, 1999). Several studies suggested that statins possess anti-inflammatory properties owing to their capacity to reduce the number of inflammatory cells in atherosclerotic plaques (Vaughan et al., 2000). Statins attenuate P-selectin expression and leukocyte adhesion in normocholesterolemic animals by increasing endothelial NO production (Lefer et al., 1999; Scalia et al., 2001).

The role of CRP as a mediator in atherosclerosis and inflammation is being investigated worldwide. Mahajan and Dhawan (2010) investigated the effect of CRP on matrix MMP-1, 2, 9 and their tissue inhibitor (TIMP-1) gene expression in THP-1 monocytic cell line. Atorvastatin was able to significantly attenuate CRP-induced MMPs expression and augmented TIMP-1 gene expression significantly. In patients in the early stages of aortic valve disease, atorvastatin at a dose of 20 mg reduced the biomarkers of inflammation, such as hsCRP, LI-6 and MCP-1 (Dimitrow and Jawien, 2010). Thongtiang et al. (2011) data have indicated that the maximum dosage of atorvastatin or rosuvastatin therapy significantly lower CRP levels but also moderately increase insulin levels. Hernandez et al. (2011) results suggested that 12-week atorvastatin is effective in reducing Lp(a) in patients with dyslipidaemia who free of CVD. In addition, this is also the first evidence that the drug increases interleukin-10 (IL-10) in a dose-dependent manner.

Periodontal disease is an independent risk factor for atherosclerosis. High-dose atorvastatin reduces periodontal and arterial inflammation, suggesting a newly documented anti-inflammatory effect of statins (Subramanian et al., 2013). Venous thromboembolism has also been shown to be associated with inflammation. A 3-days administration of atorvastatin that might reduce venous thromboembolism risk has been found to exert anti-inflammatory properties in patients at cardiovascular risk. The field of redox biology accepts that statins have antioxidant properties. Melo et al. (2013) results suggested that atorvastatin and pravastatin but
not simvastatin exhibit anti-inflammatory and antioxidant activity in endotoxin-induced acute lung injury.

Recently, in a 5-year follow-up study showed that atorvastatin in a small dose significantly reduces level of uric acid and inflammatory cytokines, improve hemodynamic parameters and improve 5-year survival in patients with dilated cardiomyopathy (Bielecka-Dabrowa et al., 2013). Atovastatin and tinidazole can reduce the expression levels of MMP-2, TNF-α and IL-1 in the rabbits with atherosclerosis and periodontitis respectively (Zhang et al., 2013). Statins inhibit MHC-II expression on ECs and monocyte-macrophages via inhibition of the promotor IV of the transactivator CIITA and thereby repress MHC-II-mediated T cell activation (Kwak et al., 2000). In addition, statins have been shown to decrease CD40 expression and CD40-related activation of vascular cells (Mulhaupt et al., 2003).

2.6.9. Statins and its effects on the myocardium

Cardiac hypertrophy is an adaptive response of the heart to pressure excess. In the myocardium, the small GTP-binding proteins, Rho, Rac, Ras and oxidative stress are concerned in the hypertrophic response (Thorburn et al., 1997). Recent animal studies suggest that a phagocyte-type NADPH oxidase may be a relevant source of ROS in the myocardium (Aikawa et al., 2001; Bendall et al., 2002). NADPH oxidase-dependent ROS production appears to be involved in cardiac hypertrophy in response to pressure excess (Li et al., 2002), stretch (Aikawa et al., 1999), angiotensin II-infusion (Nakagami et al., 2003) and α-adrenergic stimulus (Xiao et al., 2002).

Certainly, statins inhibit angiotensin II-induced oxidative stress and cardiac hypertrophy in rodents (Takamoto et al., 2001). This has also been demonstrated in clinical studies where statins inhibit cardiac hypertrophy in humans with hypercholesterolemia (Lee et al., 2002). NADPH-oxidase-mediated ROS are increased in left ventricular myocardium from patients with heart failure and associate with an increased activity of Rac1 GTPase and admittance of oral statin treatment is able to decrease Rac1 function in the human heart (Maack et al., 2003). Atorvastatin attenuates lethal reperfusion-induced injury in a manner that is reliant on PI3K and Akt activity and the presence and activity of eNOS (Bell and Yellon, 2003).
The Scandinavian Simvastatin Survival Study (4S) suggests that statins reduce the incidence and morbidity of heart failure (Kjekshus et al., 1997). Patients with heart failure are characterized by increased vascular tone and endothelial dysfunction (Drexler, 1998), which may be improved by statin therapy. Statins have proven to preserve cardiac function in animal models of myocardial hypertrophy and heart failure, such as aortic banding, MI and several transgenic models (Takemoto et al., 2001; Laufs et al., 2002). Chen et al. (2007) results provide novel in vivo evidence for the key role of Connexin43 gap junctions in left ventricular hypertrophy and the possible mechanism in anti-hypertrophic effect of statins. Treatment with atorvastatin may have beneficial effects on left ventricular hypertrophy in spontaneously hypertensive rats.

In a prospective, double blind, placebo-controlled study, in patients with dilated cardiomyopathy, symptomatic and nonischemic, were randomly separated into two groups receiving statin or placebo for 14 weeks (Node et al., 2003). Although patients receiving statins showed a modest decrease in serum cholesterol level compared to patients receiving placebo, these patients confirmed a considerable improvement in exercise endurance, as showed by a lower New York Heart Association functional class compared to patients receiving placebo. This may be enhanced left ventricular ejection fraction in the statin group, except in the placebo group.

In addition, plasma concentrations of brain natriuretic peptide (BNP), TNF-α and IL-6 and were reduced in the statin group when compared to the placebo group. The short-term statin therapy improves cardiac function, neurohormonal imbalance and symptoms related with idiopathic dilated cardiomyopathy. These interpretations were confirmed in a second study using cerivastatin (Laufs et al., 2004). These findings recommend that statins may have therapeutic benefits in heart failure patients irrespective of serum cholesterol levels or atherosclerotic heart disease.

**2.6.10. Statins and blood pressure**

Hypertension is characterized by endothelial dysfunction. Saluveer et al. (2013) observed acute statin effects in hypertension appear to be endothelium-independent and related to VSMC function. These actions may provide the beneficial pleiotropic effects of statin therapy even in the acute *in vivo* setting. In hypertensive rats, arterial blood pressure was increased about 40%
and brain enzyme activities of SOD and CAT were decreased significantly compared. Induction of hypertension significantly decreased GSH content and increased MDA level of brain tissue. Treatment with atorvastatin improved the activity of SOD and prevented from GSH decrement during hypertension and it might have saved the brain tissue of hypertensive rats from hypertension-induced oxidative stress (Mohammadi et al., 2013). The hypotensive effect of atorvastatin is associated with flow-mediated dilation improvement in hypertensive patients with normal lipid levels. Although this could be related to changes in oxidative stress and endothelial function (Tycinska et al., 2011).

2.6.11. Statins and ischemic stroke

Stroke is the rapidly developing loss of brain functions due to a disturbance in the blood vessels of brain. The FHS and the MRFIT confirmed significant relationship between ischemic stroke and cholesterol levels (Kannel et al., 1971). Large clinical trials with statins is the reduction in ischemic stroke (Crouse et al., 1998), for example, the Heart Protection Study (HPS) shows a 28% reduction in ischemic strokes in over 20,000 patients with cerebrovascular disease or other high-risk situation (Collins et al., 2004). As a result of the findings of these large statin trials raise the interesting question of how a class of cholesterol-lowering agents can reduce ischemic stroke when ischemic stroke is not associated to cholesterol levels.

Cerebrovascular tone and the flow of blood are regulated by endothelium-derived NO (Dalkara et al., 1994). Mutant mice lacking eNOS (eNOS/−) are fairly hypertensive and extend greater proliferative and inflammatory response to vascular injury (Huang et al., 1995). Certainly, eNOS/− mice develop larger cerebral infarcts following cerebrovascular occlusion (Huang et al., 1996). Therefore, the valuable effects of statins in ischemic stroke may be due to their ability to upregulate the expression and activity of eNOS (Kureishi et al., 2000). Interestingly, statins treatment did not affect BP or heart rate before, during and after cerebrovascular ischemia and did not alter levels of serum cholesterol in mice, consistent with neuroprotective properties of statins.

Additionally, to increases in cerebral blood flow, other positive effects of statins are likely to happen that can impact on the severity of ischemic stroke. Statins attenuate P-selectin expression and leukocyte adhesion via increases in NO production in a model of cardiac
ischemia and reperfusion (Lefer et al., 2001). Several other studies have reported that statins upregulate tissue-type t-PA and downregulate PAI-1 expression through a same mechanism involving inhibition of Rho geranylgeranylation (Essig et al., 1998).

    Early outcome measured by National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS) was better in acute stroke patients treated with atorvastatin than in those treated with simvastatin. These variations may reveal a neuroprotective effect unique to atorvastatin (Lampl et al., 2010). The Stroke Prevention by Aggressive Reduction of Cholesterol Levels (SPARCL) trial showed daily treatment with 80 mg of atorvastatin in patients with a recent stroke or TIA reduced the incidence of fatal or nonfatal stroke by 16% (Huisa et al., 2010). Compared with placebo, use of high dose atorvastatin (80 mg/day) for secondary stroke prevention is not only of significant clinical benefit but can also be considered cost effective. It produces significant benefits in health with an incremental cost within reasonable limits (Arrospide et al., 2010).

    Atorvastatin (20 mg/day) may be beneficial in reducing ischemic stroke recurrence in ischemic stroke patients with a history of intracranial hemorrhage and is not associated with an increased risk of intracranial hemorrhage recurrence (Jia and Zhou, 2013). Recently Ouk et al. (2013) study evidenced that the anti-inflammatory action of atorvastatin is arbitrated, by PPARα. The reduction in IL-6 plasmatic levels was PPARα dependent. The expression of the adhesion molecules ICAM-1 and VCAM molecule was reduced by the atorvastatin treatment and this effect was PPARα dependent in the cortex but not in the striatum of treated animals. Atorvastatin also reduced the cerebral expression of iNOS in the cortex, but there is no effect in the striatum of treated animals, whatever the PPARα status.

2.6.12. Statins and dementia

    Dementia is a most important public health problem because of its high occurrence in elderly individuals, mainly in the growing category of subjects aged 80 years or more. Dementia is a syndrome of chronic or progressive nature with multiple disturbances of the functions of higher cortical. This syndrome generally occurs in cerebrovascular disease, Alzheimer’s disease and in other conditions primarily or secondarily affecting the brain (Corrao et al., 2013). There is
accumulating evidence that elevated serum cholesterol may be implicated in the pathogenesis of dementia.

A nested case control study based on the United Kingdom-based General Practice Research Database established that among the individuals with 50 years and older who taken statin therapy, the risk for developing dementia was considerably reduced, independent of their lipid status (Jick et al., 2000). In addition, there is no influence on the risk of developing dementia in this population who received other lipid-lowering agents. The systemic vascular protective effects of statin treatment are expected to contribute to their beneficial effects, particularly on vascular forms of the dementia syndrome. Certainly, the results of the HPS and Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trials do not demonstrate the efficacy of statins in slowing cognitive decline and dementia (Shepherd et al., 2002).

A novel pharmacological effect of atorvastatin mediated by reducing oxidative damage may be one mechanism underlying benefits of atorvastatin in Alzheimer disease (Barone et al., 2011). Recent evidence suggests that treatment of mild-to-moderate Alzheimer's disease with atorvastatin provides significant benefit on the Alzheimer Disease Assessment Scale-Cognitive (ADAS-cog) after 6 months. A significant positive effect on ADAS-cog performance occurred after 6 months of atorvastatin therapy compared with placebo, but the level of benefit produced may be predicated on earlier treatment, an persons apolipoprotein E genotype or whether the patient exhibits elevated cholesterol levels (Sparks et al., 2006).

A population-based, nested case-control study was carried out by including the cohort of 152,729 patients from Lombardy (Italy) aged 40 years or older who were newly treated with statins. Compared with patients who had very short statins coverage (less than 6 months), those on 7-24, 25-48 and >48 months of coverage respectively had risk reductions of 15%, 28% and 25%. Simvastatin and atorvastatin were both associated with a reduced risk of dementia, while no similar evidence was noted for fluvastatin and pravastatin. It is evident that the long-term use of statins seems effective for the prevention of dementia (Corrao et al.,
Therefore, statins exert numerous positive effects through various mechanisms in the presence of atherosclerotic risk factors.