INTRODUCTION

Coronary artery disease (CAD) or atherosclerotic heart disease is the end result of the accumulation of atheromatous plaques within the walls of coronary arteries that supply the myocardium with oxygen and nutrients. CAD comprises a group of clinical presentations that include acute coronary syndrome (ACS) (unstable angina, myocardial infarction) or asymptomatic CAD, all of which represent a major cause of morbidity worldwide. Moreover, CAD is the commonest cause of sudden death all over the world in populations over age of 20 years ***(Enrique et al., 2012)***.

Chronic inflammation, oxidative stress, and endothelial dysfunction play an important role in atherosclerosis. Moreover, endothelial dysfunction has been regarded as an early event in the atherosclerotic process, and has a high predictive value for ischemic events. Some plasma biomarkers of inflammation and endothelial dysfunction have been recognized as important cardiovascular risk factors ***(Zoppini et al., 2006).***

It is widely recognized that adipose tissue is not only a reservoir for energy storage, but also an endocrine tissue that secretes various bioactive molecules called adipokines which include adiponectin, tumor necrosis factor-α (TNF-α), interleukine-6 (IL-6), and apelin ***(Yokoyama et al., 2010)***.

Apelin is a peptide that has been identified as the endogenous ligand to the angiotensin receptor-like1 (APJ) receptor which resembles the angiotensin receptor but does not bind to angiotensin II (Ang II). Apelin is expressed in human heart, lungs, kidneys, adrenal glands, adipose tissue, and large conduit vessels including coronary arteries ***(Kleinz and Darenport, 2005).*** In contrast to angiotensin, which is a potent vasopressor and anti-diuretic hormone, apelin lowers blood pressure via a nitric oxide dependent mechanism, produces diuresis by inhibition of arginine vasopressin activity and release, and has a positive inotropic effect ***(Chong et al., 2006).***

The relation between plasma apelin and chronic heart failure has been widely investigated. However, there are few studies suggesting the relation between plasma apelin and atherosclerosis. Although experimental evidence supports the inhibitory effect of apelin on atherosclerosis progression, the available human data of the antiatherogenic properties of apelin are limited ***(Li et al., 2008).***

Recent studies have indicated that apelin concentration is inversely associated with the severity and the acute phase of CAD, which suggests its involvement in the progression and destabilization of coronary plaques. In addition, adipokines seem to link adiposity, inflammation, and atherosclerosis and also provide novel therapeutic targets ***(Kadoglou et al., 2010).***

AIM of THE WORK

This study aims to investigate the plasma apelin level in patients with CAD and to clarify the relationship between atherosclerosis and apelin.

I- CORONARY ARTERY DISEASE

A) Introduction:-

Coronary artery diseases (CAD) are the most common cause of ischemic attacks, which occur when blood flow to the myocardium is interrupted ***(Antman et al., 2007)***. The myocardial ischemia occurs because myocardial blood flow fails to provide sufficient blood and oxygen to meet the myocardial demand that is required for contraction, relaxation, and cellular metabolism. This failure is commonly caused by decreased blood supply or increased myocardial demand for blood and oxygen when blood supply is fixed by obstructive CAD ***(Selwyn, 2010)***.

The clinical presentations of ischemic heart disease (IHD) include stable angina pectoris, unstable angina (UA), myocardial infarction (MI), heart failure, and sudden death ***(Findlay and Cunningham, 2005)***.

B) Epidemiology:-

On the basis of the National Heart, Lung and Blood Institute (NHLBI) and Framingham Heart Study (FHS), CAD makes up more than half of all cardiovascular events in men and women below age of 75 years**.** The life time risk of developing CAD after age of 40 years is 49% for men and 32% for women ***(Lloyd-Jones et al., 2010).***

Cardiovascular diseases (CVD) cause more deaths per year than the next four leading causes of death combined

(cancer, chronic lower respiratory diseases, accidents and diabetes mellitus) ***(Thom and Haase, 2006).***

The coronary artery disease is the most common cause of death worldwide by the year 2020**.** CAD is the most common cause of CVD deaths (45% of all CVD deaths) accounting for 7.2 million deaths/year, or 12% of all deaths worldwide **(Figure 1).** In many developed countries, CAD is the single leading cause of death ***(Beltrame et al., 2012).***

According to the Egyptian Ministry of Health (MOH) and World Health Organization (WHO) Regional office report, there is an increased prevalence of CAD in Egypt which is responsible for about 47% of all deaths among Egyptians ***(Ibrahim et al., 2009).***

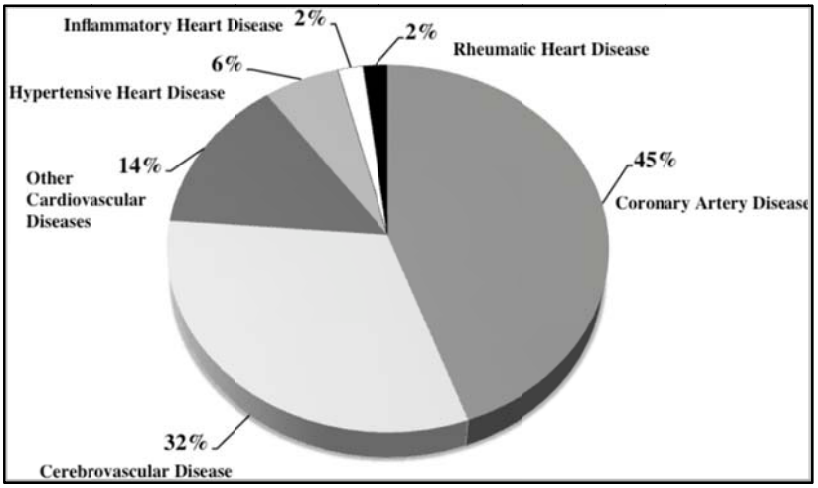

Figure (1): Distribution of cardiovascular diseases accounting for deaths worldwide in 2004 ***(Beltrame et al., 2012).***

C) Pathogenesis of CAD:-

The pathophysiology of atherosclerosis encompasses a complex interaction between endothelial cells, smooth muscle cells, platelets, and leucocytes. The vascular inflammation, lipid build up, calcium and cellular debris within the intimal walls lead to plaque formation. This contributes to vascular remodeling, luminal obstruction, abnormalities in blood flow, and reduced oxygen supply to the myocardium **(Figure 2).** Clinically, it is manifested as angina pectoris, with or without ST-segment changes on the electrocardiography (ECG) ***(Mahmood, 2009).***

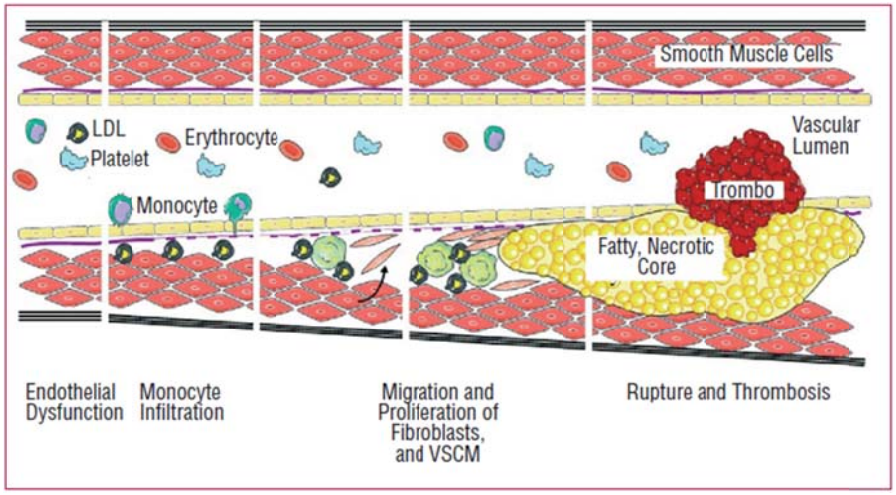

Figure (2): The atherosclerotic process. Low density lipoprotein (LDL), vascular smooth muscle cells (VSMCs) ***(Rodriguez et al., 2007).***

1) Development and Progression of Atherosclerosis:-

a) Endothelial dysfunction:-

The endothelium, once considered a mere selectively permeable barrier between the blood stream and the outer vascular wall, is now recognized to be a crucial homeostatic organ, fundamental for the regulation of the vascular tone and structure.

Under physiologic conditions, endothelial stimulation induces the production and release of nitric oxide (NO), which diffuses to surrounding tissue and cells and exerts its cardiovascular protective role by relaxing media-smooth muscle cells, preventing leukocyte adhesion and migration into the arterial wall, muscle cell proliferation, platelet adhesion and aggregation and adhesion molecule expression ***(Taddei et al., 2003).***

In disease conditions, including the presence of cardio-vascular risk factors, the endothelium undergoes functional and structural alterations, thus losing its protective role and becoming a proatherosclerotic structure. In the earliest stages, the principal endothelial alteration is merely functional and addressed as (endothelial dysfunction). The fundamental feature of this condition is the impaired NO bioavailability. This can be the consequence of either a reduced production by endothelial NO synthase (eNOS) or, more frequently, of an increased breakdown by reactive oxygen species (ROS) ***(Versari et al., 2009).***

In the presence of impaired NO bioavailability, the endothelium implements various physiological pathways in the attempt to compensate for NO deficiency. For instance, endothelium dependent vasodilation is warranted, although impaired, also in the presence of cardiovascular risk factors by the production and release of endothelium derived vasodilators other than NO, such as prostanoids and other endothelium-derived hyperpolarizing factors. Along with NO deficiency, a dysfunctioning endothelium also becomes the source of other

substances and mediators that are detrimental to the arterial wall, including endothelin-1, thromboxane A2, prostaglandin H_2, and ROS ***(Taddei et al., 2003).***

b) Lipoprotein retention and activation of immune cells:

There is growing evidence that oxidized low density lipoprotein-cholesterol (ox-LDL-C) is a key active component in the generation of atherosclerosis, rather than a passive substance that accumulates within macrophages. In the presence of elevated plasma low density lipoprotein-cholesterol (LDL-C) levels, the concentration of LDL-C within the intima is increased. The LDL-C molecule undergoes oxidative modification (peroxidation of polysaturated fatty acids) that alters its metabolism ***(Mani et al., 2010).*** Ox-LDL-C within the intima may play a role in the adhesion of circulating monocytes to the arterial wall and a potent inhibitor of the migration of macrophages out of the intima. By these two mechanisms, ox-LDL-C may serve to attract and retain monocytes and macrophages within the vessel wall ***(Min et al., 2005).***

Activated endothelial cells express leukocyte adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), causing blood cells to adhere at the sites of activation. Monocytes and lymphocytes preferentially adhere to these sites ***(Mani et al., 2010).*** Once the blood cells have attached, chemokines produced in the underlying intima stimulate them to migrate through the inter-endothelial junctions and into the sub-endothelial space ***(Hansson and Hermansson, 2011).***

c) Role of immune cells in developing plaque:-

Innate immunity is a key player in atherosclerosis, as the immune inflammatory apparatus is chronically activated, either due to the persistence of pro-inflammatory stimuli or due to the failure of regulatory mechanisms that should facilitate resolution. Significant progress has been made in the field linking innate immune sensors to the recognition of cholesterol and modified lipoproteins, The most abundant cell types within the atherosclerotic plaque are innate immune cells, such as monocyte, macrophages, dendritic cells (DCs) and mast cells **(Figure 3)** ***(Stewart et al., 2010).***

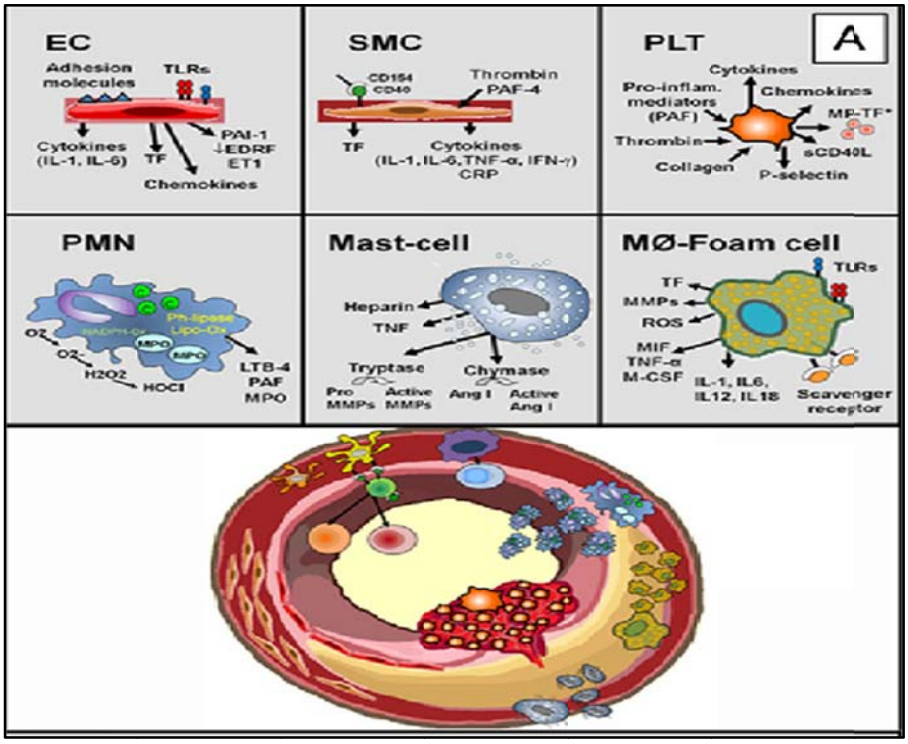

Figure (3): Role of innate and adaptive immunity in plaque instability in patients with systemic evidence of inflammation ***(Crea and Liuzzo, 2013).***

i. Role of macrophages and monocytes:-

Macrophages play a central role in the atherogenic process as modulators of both lipid metabolism and immune responses. The accumulation of cholesterol-loaded macrophages in the arterial wall is the hallmark of the early atherosclerotic lesion. Cholesterol loading of macrophages stimulates the production of inflammatory mediators, such as cytokines and ROS that recruit other cell types and contribute to the development of a complex lesion ***(Shibata and Glass, 2009).*** Within the vessel wall, monocytes undergo phenotypic modification by macrophage colony stimulating factor (M-CSF) (a powerful regulator of macrophage differentiation and proliferation) inducing them to differentiate into tissue macrophages. These macrophages are capable of expressing scavenger receptors (SRs) leading to internalization of ox-LDL-C and the creation of foam cells and fatty streaks ***(Mani et al., 2010).***

Scavenger receptors (SRs) comprise a structurally diverse group of proteins. This family of proteins has expanded to include eight different classes of membrane and soluble proteins (Class A, B, C, D, E, F, G, and H) encoded by distinct and unrelated genes ***(Stephen et al., 2010).*** SRs classes are grouped by the presence of shared structural domains; however, there is great structural diversity between the different classes. Despite this lack of sequence similarity or identity, all SRs retain the capacity to bind modified lipid particles in addition to a diverse range of polyanionic ligands of host-derived or exogenous origins ***(Plüddemann et al., 2007).***

Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRR) activated by endogenously derived

ligands that may be generated by pathogenic processes and include components of necrotic cells and modified lipoproteins ***(Shibata and Glass, 2009).***

TLRs mediate inflammatory activation. Like pathogen derived endotoxins, modified LDL-C can trigger TLR2 and TLR4 signaling through CD36 ***(Seimon et al., 2010).*** TLRs can affect atherogenesis, as signaling downstream of these receptors can elicit pro-inflammatory cytokine release, lipid uptake and foam cell formation and activate cells of the adaptive immune system ***(Cole et al., 2010).***

Although SR-A and CD36 mediate the majority of modified LDL-C uptake by macrophages in vitro, their genetic deletion has yielded varying effects on atherosclerosis, possibly reflecting differences in the genetic background, regional context or inflammatory features studied. Combined deficiency of SR-A and CD36 has implicated these receptors in athero-progression, by inducing pro-inflammatory gene expression and macrophage apoptosis, rather than foam cell formation ***(Manning-Tobin et al., 2009).***

ii. Role of dendritic cells (DCs) in T-cell activation:-

Dendritic cells (DCs) are defined as immune cells that internalize, process, and present antigen, leading to activation or suppression of T cells. Both pre-atherosclerotic susceptible regions of arteries and established atheromata are populated with cells that have DC-like properties ***(Choi et al., 2009).*** A study suggests that a population of DCs in atherosclerotic lesions originates from monocytes. In terms of functional significance,

there is some evidence that lesional DCs present antigen to and activate lesional T cells ***(Moore and Tabas, 2011).***

The plaque is an environment with a strong skew towards T helper-1 (Th1) lymphocytic responses, resulting in high levels of Interferon gamma **(**IFNγ) ***(Shalhoub et al., 2011).*** Role of adaptive immunity suggest that T-cell might play a pivotal role in coronary instability **(Figure 4).** T cells release large amounts of pro-inflammatory cytokines, in particular IFN-γ (thus activating monocytes and macrophages), and have direct cytolytic effects on endothelial cells, amplified by high-sensitivity C-reactive protein (hs-CRP), and on vascular smooth muscle cells. Apoptosis of vascular smooth muscle cells (VSMCs) has been implicated in destruction of the plaque surface ***(Crea and Liuzzo, 2013).***

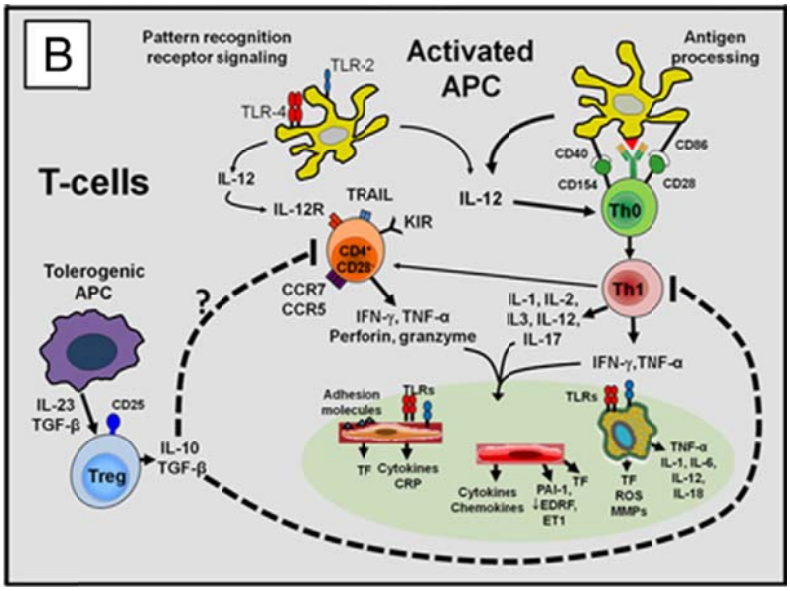

Figure (4): Role of innate and adaptive immunity in plaque instability in patients with systemic evidence of inflammation ***(Crea and Liuzzo, 2013).***

2) Main Features of Atherosclerotic Lesions:-

According to American Heart Association (AHA) Criteria for grading atherosclerotic lesions and stary's classification ***(Stray, 2000 and Mittal, 2005).*** The lesions are classified into:-

Grade 1 - Isolated intimal foamy cells (minimal change).

Grade 2 - Numerous intimal foamy cells often in layers (fatty streaks).

Grade 3 - Pools of extra-cellular lipid without a well-defined core (intermediate lesion or preatheroma).

Grade 4 - Well defined lipid core with luminal surface covered by normal intima (atheroma or fibro plaque).

Grade 5 - Lipid core with a fibrous cap with or without calcification (fibro-atheroma).

Grade 6 - Fibro-atheroma with cap defect such as haemorrhage and thrombosis.

Grade 7 - Calcification prominent.

Grade 8 - Fibrous tissue change prominent.

3) Complications of Atherosclerotic Plaque:-

The clinical complications of atherosclerosis are highly dependent on the location and size of affected vessels, the duration of the chronic process, and the type of plaque, since the severity of impairment of atherosclerosis differs throughout the vasculature ***(Mittal, 2005).*** Plaques are more commonly seen just proximal to branching points of vessels, which are areas of low shear rates. Shear rates refer to velocity gradients between the layers of fluid in close proximity to the endothelial surface **(Figure 5)** ***(Kini and Mullick, 2009).***

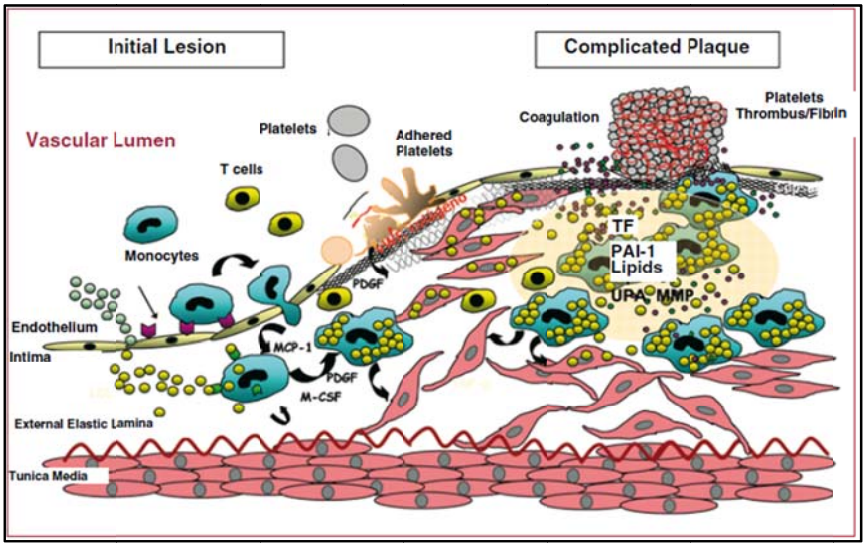

Figure (5): Schematic representation of the progression of atherosclerotic plaque from initial stages of endothelial dysfunction to advanced stages with the presence of complicated plaques ***(Badimón et al., 2009).***

Plaque's "clinical future" is based on structural information; "stable plaques" (marked by a thick fibrous cap and small lipid core) may cause pronounced arterial narrowing, yet they have less propensity to rupture. Conversely, "vulnerable plaques" (marked by a thin fibrous cap, rich lipid core, extensive macrophage infiltrate, and a paucity of smooth muscle cells) tend to be fragile, and more likely to rupture and intiate thrombosis **(Figure 6)** ***(Strom and Libby, 2011).***

Complications of atherosclerotic plaques including calcification, erosion, rupture, hemorrhage, and embolization can have dire clinical consequences due to acute restriction of blood flow or alterations in vessel wall integrity ***(Strom and Libby, 2011).***

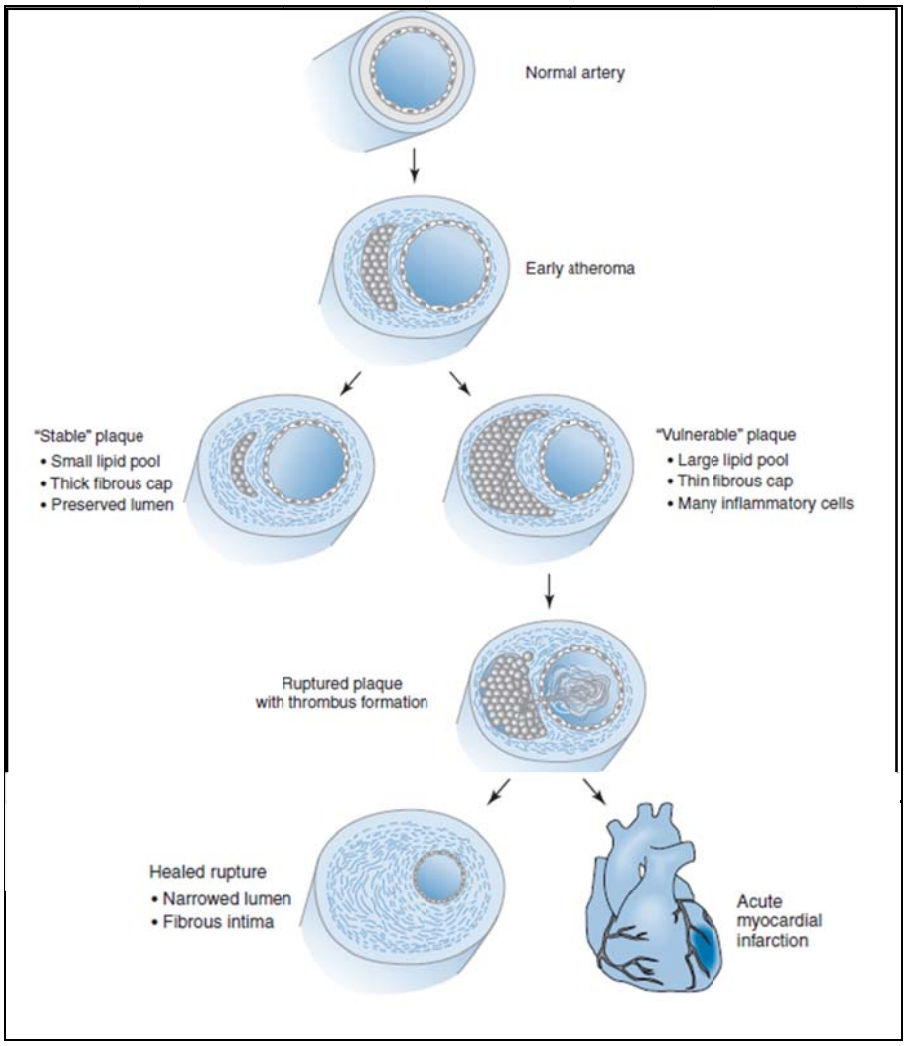

Figure (6): Stable versus vulnerable plaques ***(Strom and Libby, 2011).***

D) Risk Factors of CAD:

There is no single causative risk factor for the development of IHD. A number of genetic and environmental risk factors have been established as a cause in the development of the atherosclerotic lesion ***(Kivimaki et al., 2012).*** Many risk factors, particularly those related to lifestyle habits and