Plasma concentrations of soluble CD40 ligand in smokers with acute myocardial infarction

Thesis

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ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AHA/ACC	American Heart Association / American College of Cardiology
Akt	A serine/threonine-specific protein kinase also named Protein Kinase B
ADAM	adamalysin
Ang II	Angiotensin II
APLN	Cardio-protective factor apelin
APTT	Activated partial thromboplastin test
1ASA	Acetyl salicylic acid
BNP	Brain type Natriuritic peptide
Ca2+	Calcium
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CD	Cluster of differentiation
CD45RA+	Isoform of CD45 located on naive T cells
CD45RO+	Isoform of CD45 located on memory T cells
CK-BB	Creatine Kinase-BB
CK-MB	Creatine Kinase-MB
CK-MM	Creatine kinase-MM
CLARITY	Clopidogrel benefits MI patients receiving thrombolysis
C-Mp1	A cell-surface receptor on megakaryocytes and platelets
CNS	Central nervous system
CRP	C reactive protein
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
D	Dalton
D	Day
ECG	Electrocardiogram
ECs	Endothelial cells

EOL-3	Esinophilic leukemia cell line 3
Era	Estrogen receptor A
ERK	Extracellular signal regulated kinases 1/2
ESR	Erythrocyte sedimentation rate
ET-1	Endothelin-1
GP	Glycoprotein
HDL-C	High density lipoprotein-Cholesterol
HF	Heart failure
HMC-1	Human mast cell line-1
HMG-CoA	3-hydroxy-3-methyl-glutaryl-Coenzyme A
HR	Hour
HS-CRP	High sensitive C-reactive protein
HUVECs	Human umbilical vein endothelial cells
ICAM	Intracellular adhesion molecule
IFN-ɣ	Interferin gamma
IHD	Ischemic heart disease
IL	Interleukin
INR	International Normalization Ratio
IRF	Interferon regulating factor
ISIS	International Studies of Infarct Survival
IV	Intravenous
JAK	Janus activated kinases
Kbp	Kilo basepair
KDa	Kilo Dalton
KU812	A chronic myelogenous leukaemia cell line with a Philadelphia chromosome.
LAD	Left coronary artery disease
LBBB	Left bundle branch block
LDL-C	Low density lipoprotein-Cholesterol
LMWH	Low molecular weight heparin
LV	Left ventricle
Mac	Mitochondrial apoptosis-induced channel
MAPK	Mitogen-activated protein kinase

MCP	Moncyte chemoattractant protein
MI	Myocardial infarction
MIP	Macrophage inflammatory protein
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NF-kB	Nuclear factor kappa B
NK	Natural killer
NOS	Nitric oxide synthase
NSTE -ACS	Non ST segment elevation acute coronary syndrome
NSTEMI	Non ST segment elevation myocardial infarction
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OxLDL	Oxidized low density lipoprotein
PCI	Percutaneous coronary intervention
PDGF	Platelet-derived growth factor
PF	Platelet factor
PI3-kinase	Phosphatidylinositide 3-kinase
PKCα	Protein kinase C alpha
PMA	Phorbo-12-myristate-13-acetate
PTCA	Percutaneous transluminal coronary angioplasty
q	Short arm of the chromosome
Rac-1	Ras-related C3 botulinum toxin substrate 1
RANTES	Regulated upon activation, normal T-cell expressed, and secreted
RGD	Arginine – Glycine – Aspartate
ROS	Reactive oxygen species
Rpm	Revolutions per minute
sCD40L	Soluble CD40 ligand
SMCs	Smooth muscle cells
STAT-1	Signal transducers and activators of transcription-1
STEMI	ST segment elevation myocardial infarction
TACE	TNF-α converting enzyme
Tc	Cytotoxic T lymphocytes

TGF-β	Transforming growth factor- beta
Th0	Naïve T cells
Th	T helper cells
TIMI	Thrombolysis in myocardial infarction
TMD	Trans-membrane domain
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TPO	Thrombopoeitin
TRAF	Tumor necrosis factor(TNF)- receptor associated factor
TRITON-TIMI	Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel– Thrombolysis in Myocardial Infarction
U937	a monocyte cell line that displays many monocytes and macrophage characteristics
UFH	Unfractionated heparin
URL	Upper reference limit
VCAM	Vascular cell adhesion molecule
VEGFR-1	Vascular endothelial growth factor receptor-1
WBCs	White blood cells

Introduction

Coronary artery disease (CAD) is believed to be the single leading cause of death in both men and women in the world. Acute myocardial infarction can be defined as a rise and / or fall from a rise of cardiac biomarkers above the upper reference limit for the laboratory (URL) associated with evidence of myocardial ischemia such as chest discomfort, E.C.G. changes or radiological evidence of myocardial ischemia (***Thygesen et al., 2007).***

The rapid closure of the coronary artery by acutely formed arterial thrombi, which are composed of platelets, fibrin, and inflammatory cells, is the major cause of acute myocardial infarction (***Horii et al.,2008).***

Cardiovascular disease (CVD) is the leading cause of death in many developed countries. In 2008, CVD was directly responsible for more than 4.35 million deaths in Europe, 1.9 million of them in the European Union, accounting for 43% of all deaths in men and55% of all deaths in women. CVD may also become the leading cause of death in developing countries which currently bear 80% of its global burden ***(Erhardt, 2009).***

Smoking is a risk factor for coronary artery disease. Among persons without coronary disease who quit smoking, the risk for incident coronary disease decreases and approaches that of non-smokers within 2 to 3 years after cessation (***Rea et al., 2002).***

A large number of young people suffering from myocardial infarction (MI) are smokers (***Mohantyet al., 1998).***There are several ways in which tobacco can influence the cardiovascular system. Smoking accelerates atherosclerosis through damage to the endothelium and is associated with proinflammatory and prothrombotic responses. Post-mortem studies have provided evidence of accelerated atherogenesis in asymptomatic smokers.Tobacco smoke also increases the blood coagulability

contributing to MI (***Fitzgerald et al., 1988).*** In vivo platelet activation occurs immediately after smoking a cigarette. Smoking-induced platelet activation can be a significant contributory mechanism for acute coronary events in smokers, (***Nair et al., 2001***).

CD40 is an integral membrane protein belonging to the tumor necrosis factor (TNF) receptor superfamily. Its multipotent immunomodulating ligand, CD40L (termed gp39 or CD154), is a trimeric, type II transmembrane member of the same superfamily ***(Schonbeck and Libby, 2001).***

Originally thought to be restricted to B lymphocytes, denderitic cells, and basal epithelial cells, then CD40 was known to be present on a wide array of cells, both atheroma and non-atheroma associated. Its ligand, CD40L, was first identified in activated CD4+ T cells, mast cells, poly-morphonuclear granulocytes, and natural killer cells. Subsequent studies revealed functional CD40L expression in a wide variety of cells, namely the endothelial cells, smooth muscle cells, macrophages, and platelets ***(Anand et al., 2003).***

The role of CD40/CD40 ligand (CD40L) in atherothrombosis is widely accepted. However, the exact mechanisms linking the CD40/CD40L system and the soluble f'orm of CD40 ligand (sCD40L) with atherothrombosis are currently a topic of intensive research. Soluble CD40L has multiple autocrine, paracrine, and endocrine actions, and it may trigger key mechanisms participating in atherothrombosis ***(Antoniades et al., 2009).***

Over the recent years, it has been well established that interactions between CD40 and its immunomodulating ligand (CD40L), expressed in a variety of cell types including platelets, vascular wall cells, and immune cells, are actively involved in atherogenetic and thrombotic mechanisms

and may serve as a link between inflammation, atherosclerosis, and thrombosis***(Antoniades et al., 2009).***

From all of the above, the pivotal role of platelet activation in atherothrombosis has made CD40L an interesting subject in the setting of cardiovascular disease and MI ***(Kayrak et al., 2011).***

Aim of the work

The aim of this work is to evaluate levels of sCD40L in smokers with acute MI versus non-smokers to identify the patients who are likely to benefit from treatment with the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonists.

Acute coronary syndromes

Acute coronary syndromes describe a spectrum of clinical syndromes ranging from unstable angina to NSTEMI (Non ST segment elevation myocardial infarction) and STEMI (ST segment elevation myocardial infarction). Patients presenting with ACS (Acute coronary syndromes) are divided into those with ST elevation (lasting ≥20 minutes) or new left bundle branch block, and those with NSTE-ACS (Non ST elevation acute coronary syndromes) which includes transient ST elevation (lasting <20 minutes), unstable angina and NSTEMI ***(Aroney et al., 2000)***.

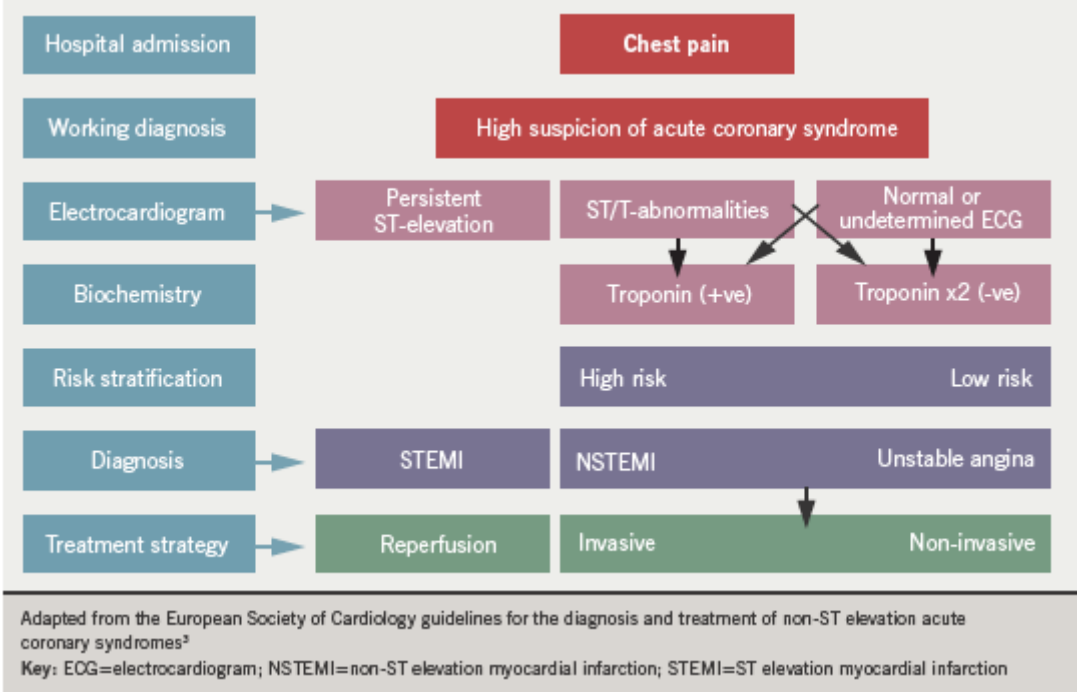

Fig (1): spectrum of acute coronary syndrome ***(Bassand et al., 2007).***

Risk factors for CAD:

Risk factors for CAD include conventional risk factors as smoking, hypertension, dyslipidemia, diabetes mellitus, obesity, mental stress and depression.

1-Smoking:

Other than advanced age, smoking is the single most important risk factor for coronary artery disease. Smoking has a particularly large impact in the developing world and annually accounts for 1.17 million deaths worldwide ***(Ezzati et al., 2005)***.

IHD (Ischemic heart disease) risk increases continuously with daily cigarette smoking. The effects of tobacco smoking on the cardiovascular system are multiple and reinforce each other and include platelet activation, endothelial dysfunction, inflammation, altered lipid levels and metabolism, and hemodynamic effects. These effects occur rapidly, often within minutes of active or passive smoking ***(Ling and Glantz, 2004)***.

In a major overview, smoking cessation was found to reduce coronary heart disease mortality by 36 % as compared with mortality in subjects who continued smoking, an effect that did not vary by age, gender, or country of origin ***(Critchley and Capewell, 2003)***.

2-Hypertension:

Hypertension is a major cardiovascular risk factor that directly contributes to coronary artery disease, stroke, congestive heart failure and renal failure. Isolated systolic hypertension, in particular, has at least as much importance as diastolic blood pressure for the outcomes of total cardiovascular mortality and stroke ***(Chaudhry et al., 2004)***.

3-Dyslipidemia:

High cholesterol levels consistently predict risk of future cardiovascular events in human populations ***(Libby, 2005)***.

Cardiovascular risk was found to be positively associated with Low density lipoprotein-cholesterol (LDL-C) levels and inversely with High

density lipoprotein-Cholesterol (HDL-C) levels. The relationship between fasting serum triglycerides and cardiovascular risk has been confounded by the inverse association between triglycerides (TG) and HDL-C, as well as by the association of triglycerides with other risk factors such as diabetes mellitus and body mass. However, triglyceride levels are considered an important independent predictor of cardiovascular risk ***(Glass and Witztum, 2001)***.

4-Diabetes mellitus:

Both type I and type II diabetes are powerful and independent risk factors for coronary artery disease, stroke and peripheral arterial disease ***(Beckman et al., 2002)***.

In type I diabetes, atherosclerosis occurs earlier in life, more diffuse, and leads to higher case fatality and shorter survival ***(Libby, 2005)***. The risk increases rapidly after the age of 40, and by the age of 55 years, 35% of patients with type I diabetes die of CAD (Coronary artery disease). The protection from CAD observed in non diabetic women is lost in women with type I diabetes ***(Laing et al., 2003)***.

The relative risk of CVD (Cardiovascular disease) in type II diabetes compared to the general population is increased two to four folds ***(Stamler et al., 1993)***.

5- Obesity and metabolic syndrome:

The metabolic syndrome represents a constellation of several established and emerging risk factors predisposing to CVD and its complications. It is well established that its key components are abdominal obesity, atherogenic dyslipidemia, hypertension, glucose intolerance, and proinflammatory and prothrombotic states ***(Eckel et al., 2005)***.

6-Mental stress and depression:

Both depression and mental stress predispose to increased vascular risk. The adrenergic stimulation of mental stress can augment myocardial oxygen requirements and aggravate myocardial ischemia. Mental stress can cause coronary vasoconstriction, particularly in atherosclerotic coronary arteries, and hence can influence myocardial oxygen supply as well. Studies have further linked mental stress to platelet and endothelial dysfunction, the metabolic syndrome, and the induction of ventricular arrhythmias ***(Kivimaki et al., 2002)***.

Pathology

The major cause of acute myocardial infarction is coronary atherosclerosis with superimposed luminal thrombus, which accounts for more than 80 percent of all infarcts. Myocardial infarctions resulting from nonatherosclerotic diseases of the coronary arteries are rare ***(Ohtani et al., 2006)***.

During the natural evolution of atherosclerotic plaque, especially that which is lipid laden, an abrupt and catastrophic transition can occur, characterized by plaque disruption ***(Ohtani et al., 2006)***. Disruption of plaques is considered to underlie most acute coronary syndrome ***(Boersma et al., 2003).*** Some patients have a systemic predisposition to plaque disruption that is independent of traditional risk factors ***(Wasserman and Shipley, 2006).*** Plaques that rupture or fissure tend to have a thin fibrous cap, high lipid content, few smooth muscle cells and a high proportion of macrophages and monocytes ***(Libby, 1995).***

Plaque disruption exposes substances that promote platelet activation and aggregation, thrombin generation and ultimately thrombus formation ***(Ertl and Frantz, 2005).***

The resultant thrombus interrupts blood flow and leads to an imbalance between oxygen supply and demand. If an occlusive thrombus forms, patients may develop an acute ST-segment elevation MI unless the subtended myocardium is richly collateralize ***(Thygesen et al., 2007).***

ST segment elevation myocardial infarction

Definition: Detection of rise and/or fall from a rise of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit(URL) together with evidence of myocardial ischemia which defined as any symptoms of ischemia, ECG (electrocardiogram changes indicative new ischemia, development of pathological Q waves in the ECG or imaging evidence of infarction (***Thygesen et al., 2007)***.

Included in the definition was sudden cardiac death with evidence of myocardial ischemia (new ST-segment elevation , left bundle branch block (LBBB) or coronary thrombus), biomarker elevation > 3 X URL for post PCI (Percutaneous coronary intervention) patients, or 5 X URL for post CABG (Coronary artery bypass grafting) patients. Documented stent thrombosis was recognized in the definition (***Thygesen et al., 2007)***.

Criteria for prior MI

Any one of the following criteria meets the diagnosis for prior MI:

- Development of new pathological Q waves with or without symptoms.
- Imaging evidence of a region of loss of viable myocardium that's thinned & fails to contract in the absence of non ischemic cause.
- Pathological finding of healed or healing MI (Myocardial infarction) (***Thygesen et al., 2007*****)** .

Incidence: The true incidence of acute MI is unknown. It is estimated that Acute MI is the leading cause of death in the North America & Europe. In

the United States, the annual death from coronary heart disease is higher than 800,000. An American has acute MI every 25 seconds **(*Rosamond et al., 2007*)**.

Diagnosis of STEMI:

History:

Despite advances in the laboratory detection of STEMI, the patient's history remains crucial to establish a diagnosis. The history should include:

1- History of risk factors for CAD and history of previous IHD.

2- **Symptoms:** The classic symptoms of MI include:

prodromal symptoms:* The prodrome is usually characterized by chest discomfort, resembling classic angina pectoris, but it occurs at rest or with less activity than usual and can therefore be classified as unstable angina. A feeling of general malaise or frank exhaustion often accompanies other symptoms preceding STEMI *(Stein and Levin, 1998)***.

Chest pain:* The classic symptom of MI is intense, durable, excruciating chest pressure, with an impending sense of doom and radiation of the pain to the left arm. However, the other symptoms of chest heaviness or burning, radiation to the jaw, neck, shoulder, back, or both arms may be encountered. The pain is prolonged, usually lasting for more than 20 minutes and frequently for a number of hours. The pain is usually retrosternal in location. The pain of STEMI may begin in the epigastrium and simulate a variety of abdominal disorders *(Stein and Levin, 1998)***.

**Atypical presentation of MI:* Atypical presentations of STEMI include the following: dyspnea without pain; classic angina pectoris without a particularly severe or prolonged episode; atypical location of the pain; CNS

(Central nervous system) manifestations, resembling those of stroke, secondary to a sharp reduction in cardiac output in a patient with cerebral arteriosclerosis; apprehension and nervousness; sudden mania or psychosis; syncope; overwhelming weakness; acute indigestion; and peripheral embolization ***(Thygesen et al., 2012)***.

Investigations:

1-ECG:

Initial ST-segment elevation or a LBBB pattern is strongly correlated with an acute occlusive obstruction of an epicardial vessel and suggests potential benefit from reperfusion therapy ***(Menown et al., 2000)***. The presence of ST-segment elevation is the principal feature that denotes current of injury and should be associated with reciprocal depression in contra-lateral leads ***(Menown et al., 2000)***.

2-Cardiac biomarkers:

A- <u>Cardiac Troponins (cTn):</u>

Troponin is localized primarily in the myofibrils (94-97 %) with a smaller cytoplasmic fraction (3-6 %). Three Troponin subunits form a complex that regulates the interaction of actin and myosin and thus regulates the cardiac contraction. The three Troponins are troponin C (calcium binding component), troponin I (inhibitory component), and troponin T (tropomyosin binding component) ***(Jaffe et al., 2006).***

Cardiac troponin subunits I and T have different amino acid sequences encoded by different genes, and are different from the predominant troponins found in other muscle such as skeletal muscle. Human cTnI has an additional post-translational 31-amino acid residue on the amino terminal end compared with skeletal muscle ***(White, 2011).***

Clinical Significance of Cardiac Troponin I & T:

1-Diagnosis of AMI:

In 2000 a joint committee of the European Society of Cardiology and the American College of Cardiology (ESC/ACC) issued new criteria that acknowledged that elevations in biomarkers were fundamental to the diagnosis of acute MI because symptoms may be atypical or non-existent and electrocardiogram changes may be absent or non-specific. By this time, cardiac troponin had replaced CK-MB as the biomarker of choice for the detection of cardiac injury ***(Alpert et al., 2000).***

To avoid false positive results, the 99th percentile should be used as the cut-off value for diagnosing AMI. The use of 2 cut-off values, one to define infarction and a second designation for unstable angina with some degree of myocardial necrosis had been also suggested ***(Apple et al., 2012).***

The 99th percentile should be measurable with an imprecision (coefficient of the variation) of 10% or less to avoid analytically false positive results ***(Jaffe et al., 2010).***

2- Prognostic value of troponins:

Elevated troponin levels on admission are of value for patients with STEMI. Regardless of therapy, an elevated troponin level is an independent predictor of death at 30 days and during long-term follow up. This is because elevations predict incomplete epicardial and more severel impaired myocardial perfusion despite normal epicardial flow ***(Mills et al., 2011).***

B- **Creatine kinase (CK):**

Cytoplasmic CK is a dimer, composed of M and/or B subunits, which associate forming the following isoenzymesCK-3 (CK-MM) predominant in both heart and skeletal muscle, CK-1 (CK-BB) which is the predominant form in the brain and smooth muscle and CK-2 (CK-MB). CK-MB is sometimes called the cardiac isoenzyme as 10-20% of the total CK activity in myocardium is from CK-MB, where as in skeletal muscles this percentage ranges from than less than 2 to 5% ***(Dekker et al., 2010).***

Clinical significance of CK-MB:

1-Diagnosis of AMI

Serum total CK activity and CK-MB concentration rise following myocardial injury. Serum CK-MB is considerably more specific for myocardial damage than is serum total CK, which may be elevated in many conditions where skeletal muscle is damaged. Consequently, CK should not be used for the diagnosis of myocardial injury unless used in combination with other more specific cardiac markers ***(Apple et al., 2003).***

The diagnostic cut-off designated at 99^{th} percentile of a reference population is assay dependent because of a lack of CK-MB standardization among manufacturers ***(Jaffe et al., 2006).***

2-Estimation of the infarct size:

CK-MB is used not only for diagnosis of MI, but they can also be used to estimate the infarct size. Several studies showed that in general association of large infarct size & worse clinical outcomes including mortality is significant & similar using multiple observed & derived measures of infarct size based on CK-MB ***(Thygesen et al., 2012).***

C- <u>Myoglobin:</u>

Myoglobin is an Oxygen binding protein of cardiac and skeletal muscle with a molecular weight of 17,800 Da. It is rapidly released from infarcted

area over some limited time and rapidly transported to serum ***(Morrow et al., 2007).***

Table (1): Advantages and disadvantages of different cardiac markers.

Marker	Advantages	Disadvantages
CK-MB	Rapid Cost effective Detected early in infarctio	Loss of specificit with skeletal muscle damage. Detection after 6 hours of myocardial necrosis.
Myoglobin	Highly sensitive Early detection of MI, within 2 hours Detects reperfusion Most useful in ruling out MI	Low specificity with skeletl muscle injury Rapid return to normal
Troponins	Powerful tool for risk stratification. Greater sensitivity and specificity than CK-MB. Detects recent MI up to 2 weeks.	Low sensitivity in MI of less than 6 hours Require repeat measures at 8-12 hours if first result is negative Less lable to detect late, minorMIs

(Morrow et al., 2007)

Table (2) Biomarkers for the evaluation of patients with STEMI

Biomarker	Molecular Weight (D)	Range of Times to Initial Elevation (hr)	Mean Time to Peak Elevations (Non-reperfused)	Time to Return to Normal Range
Frequently Used in Clinical Practice				
MB-CK	86,000	3–12	24 hr	48-72 hr
cTnI	23,500	3–12	24 hr	5-10 d
cTnT	33,000	3–12	12 hr-2 d	5-14 d
Infrequently Used in Clinical Practice				
Myoglobin	17,800	1–4	6-7 hr	24 hr
BB-CK	86,000	2–6	18 hr	Unknown
MM-CK	86,000	1–6	12 hr	38 hr

(Antman et al., 2006)

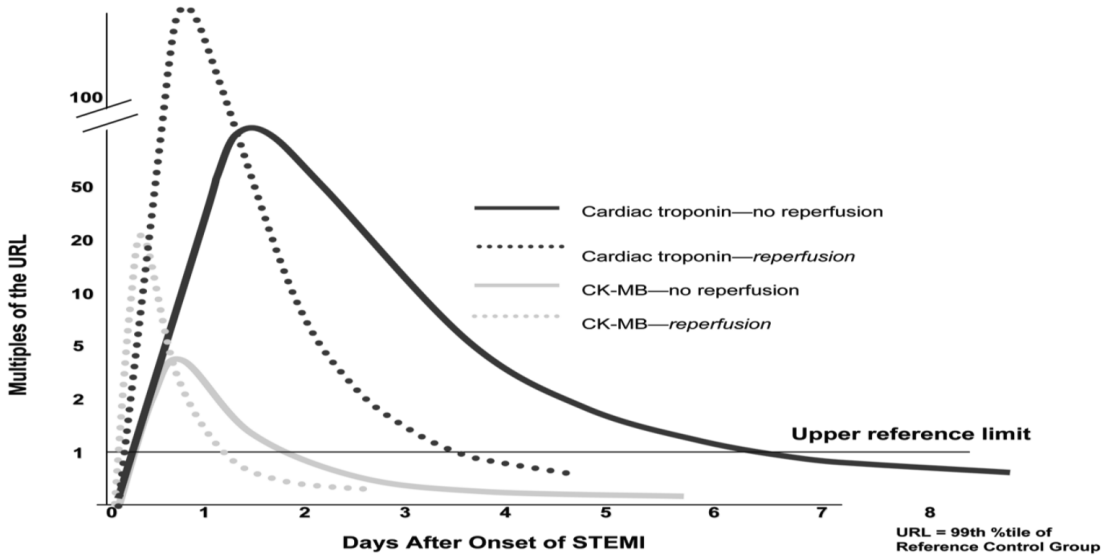

Fig (2): Cardiac biomarkers in STEMI ***(Alpert et al., 2000).***

3- Other laboratory measurements:

****Serum lipids*****:**

These are often determined in patients with STEMI. However, the results may be misleading because numerous factors that can alter the values are operating at the time of the patient's admission to the hospital. Serum triglycerides are affected by caloric intake, intravenous glucose, and recumbency ***(Wolfrum et al., 2003)***.

A lipid profile should be obtained on all STEMI patients who are admitted within 24 to 48 hours of symptoms. The success of lipid-lowering therapy in primary and secondary prevention studies and that hypolipidemic therapy improves endothelial function and inhibits thrombus formation indicate that early management of serum lipids in patients hospitalized for STEMI is advisable. For patients admitted beyond 24 to 48 hours, more accurate determinations of serum lipid levels are obtained about 8 weeks after the infarction has occurred ***(Elliott et al., 2008)***.

****WBCs:***

The elevation of the white blood cell count usually develops within 2 hours after the onset of chest pain, reaches a peak 2 to 4 days after infarction, and returns to normal in 1 week. An epidemiological association has been reported, indicating a worse angiographic appearance of culprit lesions and increased risk of adverse clinical outcomes the higher the white blood cell count is at presentation with an acute coronary syndrome ***(Kloner, 2006)***.

***ESR:**

ESR is usually normal during the first day or two after infarction, even though fever and leukocytosis may be present. It then rises to a peak on the fourth or fifth day and may remain elevated for several weeks. The increase in the ESR does not correlate well with the size of the infarction or with the prognosis ***(Kloner, 2006)***.

***CRP:**

CRP is a member of the pentraxin family. It comprises 5noncovalently associated pentamers arranged symmetrically around a central pore and has a molecular weight of 118 000Da. HS-CRP is a very sensitive measuring of CRP concentration by ELISA method ***(Honarmand et al., 2011).***

CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increasment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophagesas well as adipocytes. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6-12 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause) ***(Pepys and Hirschfield, 2003).***

Another research suggested that patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension and cardiovascular disease. A study of over 700 nurses showed that those in the highest quartile of trans fat consumption had blood levels of CRP that were 73% higher than those in the lowest quartile ***(Pradhan et al., 2001).***

An elevated High sensitive C-reactive protein (HS-CRP) level appears to identify patients presenting with STEMI, with worse angiographic appearance of the infarct artery and a greater likelihood of developing heart failure ***(Honarmand et al., 2011)***

****Hemoglobin:***

Cardiovascular mortality increases progressively as the presenting hemoglobin value falls below 14 to 15gm/dl; conversely, it also rises as the hemoglobin level increases above 17gm/dl. The increased risk from anemia probably relates to diminished tissue delivery of oxygen, while the increased risk with polycythemia may be related to an increase in blood viscosity ***(Sabatine et al., 2005a)***.

4-Echocardiography:

In patients with chest pain compatible with MI but with a non diagnostic ECG, the finding on echocardiography of a distinct region of disordered contraction can be helpful diagnostically because it supports the diagnosis of myocardial ischemia. Areas of abnormal regional wall motion are observed almost universally in patients with MI ***(Cheitlin et al., 2003)***.

5-Angiography:

On occasion, even with all of the tools outlined, the diagnosis is uncertain. This may be the result of atypical symptoms and an ECG that is difficult to interpret. In a patient in whom reperfusion therapy is contemplated, an approach that can rapidly establish the diagnosis is emergency coronary angiography ***(Simes et al., 1995)***.

6-Computed tomography (CT):

It can detect left ventricular aneurysms, and, of particular importance in patients with STEMI, intracardiac thrombi can be identified so although

cardiac computed tomography is a less convenient technique, it probably is more sensitive for thrombus detection than is echocardiography ***(Finn and Antman, 2003)***.

Management:

Management of STEMI includes reperfusion, routine general measures, anti- ischemic measures, antiplatelets, anticoagulants and other drugs.

A-Routine general measures:

Patients with STEMI should rest in bed. Progression of activity should be individualized depending on the patient's clinical status, age, and physical capacity ***(Elliot et al., 2008)***.

1- Oxygen:

It has become universal practice to administer oxygen to virtually all patients suspected of having acute ischemic-type chest discomfort ***(Elliott et al., 2008).***

2- Analgesia and control of cardiac pain:

Analgesia is an important element of management of STEMI patients in the emergency department ***(Thygesen et al., 2007)***.

B-Reperfusion:

1-Fibrinolytic therapy:

It has been well established that fibrinolytic therapy provides a survival benefit for patients with STEMI, based on large Well-controlled clinical trials ***(AIMS Trial Study Group, 1990)***. The mechanisms of benefit, which may have different time dependencies, include salvage of myocardium with reduced infarct size, favorable effect on infarct healing and myocardial

remodeling, and reduced electrical heterogeneity and potential for life-threatening ventricular arrhythmia ***(Lamas et al., 1995)***.

2- PCI (Percutaneous coronary intervention):

PCI is a very effective method for re-establishing coronary perfusion and is suitable for at least 90% of patients ***(Keeley et al., 2003)***.

3- Surgical reperfusion:

The basis for recommending surgery in emergency circumstances is the documented benefit of CABG for severe multivessel disease or left main coronary artery stenosis, particularly with reduced LV function ***(Eagle et al., 2004)***.

C- Anti ischemic therapy:

1- Nitrates.

AHA/ACC 2007 STEMI guidelines stated that sublingual or intravenous nitroglycerin is indicated for relief of ongoing ischemic discomfort, control of hypertension, or management of pulmonary congestion ***(Elliott et al., 2008)***.

2-Beta-blockers:

These drugs relieve pain, reduce the need for analgesics in many patients and reduce infarct size and life-threatening arrhythmias. ***(Sabatine, 2005)***.

D- Antiplatelet therapy:

***1- Aspirin*:**

This agent is not only useful for the primary prevention of vascular events but is also effective across the entire spectrum of acute coronary

syndromes and forms part of the initial management strategy for patients with suspected STEMI. ***(ISIS-2 Collaborative Group, 1988).***

2- Clopidogrel:

Many trials have addressed the addition of clopidogrel to aspirin, as compared to aspirin alone, in the setting of STEMI. In the CLARITY (Clopidogrel benefits MI patients receiving thrombolysis) trial of 3,491 patients with a hybrid angiographic and clinical composite endpoint, there was a 36% reduction of an occluded infarct artery at early angiography, death or recurrent MI ***(Sabatine et al., 2005b)***.

3- ***Prasugrel***:

It has a more potent antiplatelet effect than clopidogrel. The use of prasugrel as compared to clopidogrel results in a significant reduction in the primary efficacy endpoint of death from cardiovascular causes, non fatal MI or non fatal stroke **(*Wiviott et al., 2007*)**.

4- Glycoprotein IIb/IIIa Inhibitors:

Beyond aspirin and clopidogrel, there has been intensive study of the use of intravenous IIb/IIIa inhibitors in the setting of reperfusion therapy. With primary PCI, there is clear evidence from five randomized trials that adding GPIIb/IIIa inhibitors will reduce the composite of death, re-infarction, and repeat target vessel revascularization ***(Antoniucci et al., 2003)***.

Another Meta analysis of the use of abciximab in STEMI confirms the benefit of IIb/IIIa inhibition in conjunction with primary PCI (but not with thrombolysis) ***(De Luca et al., 2005)***.

E-Other drugs:

***Statins:**

With the marked salutary effects on long-term survival, prevention of re-infarction, and subsequent coronary revascularization of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase drugs, it is important that a cholesterol panel (total, low-density lipoprotein, and high-density lipoprotein) be obtained on admission and no later than the first 24 hours. Trials of patients with acute coronary syndromes have reinforced the benefit of early initiation of statins in patients with acute MI ***(Antman et al., 2006)***.

Non-STsegment elevation myocardial infarction

Epidemiology and natural history:

The diagnosis of NSTEMI is more difficult to establish than STEMI and therefore its prevalence is harder to estimate. Overall data suggest that the annual incidence of NSTEACS is higher than that of STEMI. The ratio between NSTE-ACS and STEMI has changed over time, as the rate of NSTE-ACS increased relative to STEMI, without any clear explanation for the reasons behind this evolution. This change in the pattern of NSTE-ACS could actually be linked to changes in the management of disease and greater efforts in prevention of CAD over the last 20 years ***(Thygesen et al., 2012).***

Clinical presentation:

1- History:

The 5 most important factors derived from the initial history that relate to the likelihood of ischemia due to CAD are 1) the nature of the anginal symptoms, 2)prior history of CAD, 3) sex, 4) age, and 5) the number of traditional risk factors present ***(Pryor et al., 1993).***

a- Anginal symptoms:

The typical clinical presentation of NSTEMI is retrosternal pressure or heaviness radiating to the left arm, neck or jaw, which may be intermittent (usually lasting several minutes) or persistent. These complaints may be accompanied by other symptoms such as diaphoresis, nausea, abdominal

pain, dyspnea and syncope. However, atypical presentations are not uncommon ***(Canto et al., 2002)*.**

b- Past history of CAD:

Approximately 80 % of patients have a history of cardiovascular disease and most have evidence of prior coronary risk factors ***(Khot et al., 2003)*.**

c- Age &sex difference:

Women present more often with unstable angina, comprising 30-45% of patients with unstable angina in several studies compared with 25 to 30% of patients with NSTEMI and approximately 20% of patients with STEMI. The outcomes are similar for men and women with NSTEMI ***(Scirica et al., 1999)*.**

2-Investigations:

1-ECG:

The resting 12-lead ECG is the first-line diagnostic tool in the assessment of patients with suspected NSTEMI. It should be obtained within 10 min after first medical contact upon arrival of the patient in the emergency room and immediately interpreted by a qualified physician ***(Diercks et al., 2006)*.**

In NSTEMI, ST depression (or transient ST elevation) and T wave changes occur in up to 50% of patients ***(Kleiman et al., 2002)*.** New (or presumably new) ST segment deviation is a specific and important measure of ischemia and prognosis ***(Holmvang et al., 2003)*.**

2-Cardiac biomarkers:

With the use of troponins, which are more sensitive than CK-MB, a greater percentage of patients are classified as having NSTEMI, which is associated with a worse prognosis ***(Horwich et al., 2003)*.**

Short- and long-term studies have shown that troponin levels correlate with the risk of death and the combined risk of death/MI ***(Ottani et al., 2000)***; with a clear gradient of risk as troponin levels increase ***(Heeschen et al., 1999)*.**

Angiographic studies have shown that evidence of thrombus; complex lesions and a reduced TIMI flow grade were more common in patients with elevated troponin levels than in those with normal levels ***(Diderholm et al., 2002)*.**

****Markers of inflammatory activity***

There are various inflammatory markers that have been investigated over the past decade for patients with NSTEACS such as CRP, serum amyloid A and interleukin-6 ***(Morrow et al., 2000)*.**

C-reactive protein measured by high-sensitive (hsCRP) assays is the most widely studied and linked to higher rates of adverse events ***(James et al., 2003)*.**

****Neuro hormonal markers:***

B-type natriuretic peptide (BNP) is a 32 amino-acid peptide that is released predominantly from ventricular myocardium in response to increased ventricular wall stress. BNP is produced as a pro-hormone that is cleaved toward the N-terminal to produce BNP and the terminal portion, NT-proBNP ***(Morrow and Braunwald, 2003).***

Both brain-type (B-type) natriuretic peptide (BNP) or its N-terminal prohormone fragment (NT-proBNP), are highly sensitive and fairly specific markers for the detection of LV dysfunction. BNP levels correlate with left

ventricular pressure, and increase in response to myocardial stretching in the event of myocardial ischemia ***(Wiese et al., 2000)*.**

There are robust retrospective data in NSTE-ACS showing that patients with elevated BNP or NT-proBNP levels have a three- to five-fold increased mortality rate when compared with those with lower levels. The level is strongly associated with the risk of death even when adjusted for age, Killip class and LV ejection fraction ***(Jernberg et al., 2002)*.**

Values taken a few days after onset of symptoms seem to have superior predictive value when compared with measurements on admission ***(Weber et al., 2006)*.** So they are markers of long-term prognosis, but have limited value for initial risk stratification and hence for selecting the initial therapeutic strategy in NSTE-ACS ***(Mueller et al., 2006)*.**

3- Imaging modality:

****Echocardiography:***

LV systolic function is an important prognostic variable in patients with ischemic heart disease and can be easily and accurately assessed by echocardiography. In experienced hands, transient localized hypokinesia or akinesia in segments of the left ventricular wall may be detected during ischemia, with normal wall motion on resolution of ischemia ***(Cheitlin et al., 2003)*.**

****Coronary Angiography*:**

Coronary angiography should be planned as soon as possible (urgent invasive strategy) in patients with severe ongoing angina, profound or dynamic ECG changes, major arrhythmias, or haemodynamic instability upon admission or thereafter. These patients represent 2-15% of the patients admitted with NSTE-ACS ***(Yan et al., 2006)*.**

Management of NSTEMI:

1-General measures:

Patients with rest pain and ECG changes within the previous 48 hours should be admitted to hospital. Antithrombotic and anti-ischemic therapy should be commenced without delay when the patient is first seen in the emergency department, chest pain unit, or coronary care unit. Patients should be placed on bed rest, ideally with continuous ECG monitoring for arrhythmias and ischemia ***(Bertrand et al., 2000)***.

2-Anti ischemic agents:

A-Nitrates:

Nitrates are endothelium-independent vasodilators that increase myocardial blood flow by coronary vasodilation and also reduce myocardial oxygen demand ***(Anderson et al., 2007)***.

B- Beta blockers:

Beta blockers are recommended to be initiated orally, in the absence of contraindications (e.g., heart failure), within the first 24 h ***(Ellis et al., 2003)***.

3-Antiplatelet agents:

The most commonly used are aspirin, thienopyridine group (clopidogrel and ticlopidine) and glycoprotein IIb/IIIa inhibitors.

A-Aspirin:

It is recommended that Acetyl salisylic acid (ASA) be initiated as soon as the diagnosis of ACS is made or suspected unless contraindicated and that it be continued indefinitely***(Anderson et al., 2007)***.

B-Thienopyridine group:

Clopidogrel is a thienopyridine derivative that inhibits platelet aggregation, increases bleeding time, and reduces blood viscosity by inhibiting adenosine diphosphate (ADP) action on platelet receptors ***(Storey et al., 2005)***.

C- Glycoprotien IIb/IIIa inhibitors:

The GP IIb/IIIa inhibitors prevent the final common pathway of platelet aggregation, the fibrinogen-mediated cross linkage of platelets. These agents inhibit platelet aggregation caused by all types of stimuli (e.g. thrombin, ADP, collagen, serotonin and others). Three GP lIb/Illa inhibitors have been approved for clinical use, namely abciximab, eptifibatide, and tirofiban ***(SYMPHONY Investigators, 2000)***.

The benefit of GP IIb/IIIa inhibition appears greater when used in high-risk patients. Patients with ST segment changes have a greater absolute benefit as compared with patients without ST changes ***(Boersma et al., 2002)***.

Diabetic patients with NSTE-ACS had a 26 % reduction in mortality with GP IIb/IIIa inhibition as compared with no reduction in mortality in nondiabetics ***(Roffi et al., 2001)***. Also the benefit of GP IIb/IIIa inhibition appears to be greatest in patients at high risk as identified by an elevated troponin level ***(Peterson et al., 2003)***.

The benefit of GP IIb/IIIa inhibition has been confirmed even on a background of clopidogrel pre-treatment ***(Kastrati et al., 2006)*** and was seen in high-risk patients with or without revascularization ***(Stone et al., 2007)***.

These subgroups have more thrombus at coronary angiography and thus are at risk for microvascular embolization***(Wong et al., 2002).***

Opinion is divided on the optimal timing of GP IIb/IIIa inhibition, with some advocating use of GP IIb/IIIa inhibition at the time of presentation, whereas others reserve it for use during PCI. The ACC/AHA Guidelines note that either strategy is acceptable. Starting therapy in the emergency department yields early benefit, which is supported by observational studies that have found a 10 % lower mortality among patients treated early ***(Peterson et al., 2003)***.

Results from a randomized trial of these two strategies failed to demonstrate a difference in recurrent ischemic events, and noted a higher rate of bleeding in the broader-based, emergency department use strategy ***(Stone et al., 2007)***.

4-Anti coagulants:

Anticoagulants are used in the treatment of NSTE-ACS to inhibit thrombin generation and/or activity, thereby reducing thrombus-related events. There is clear evidence that anticoagulation is effective in addition to platelet inhibition and that the combination of the two is more effective than either treatment alone ***(Harrington et al., 2004)***

A-Heparin:

Anticoagulation, traditionally with unfractionated heparin (UFH) is a cornerstone of therapy for patients with NSTEMI ***(Theroux et al., 1988)***.

B-Low molecular weight heparins:

LMWH (plus aspirin) has proved effective compared with aspirin alone, leading to a 66% reduction in the odds of death or MI ***(Eikelboom et al., 2000)***.

C-Fondaparinux:

The only selective factor-Xa inhibitor available for clinical use is fondaparinux which is a synthetic pentasaccharide that is an indirect Xa inhibitor that requires anti-thrombin for its action ***(Yusuf et al., 2006***).

D-Direct thrombin inhibitors:

A meta-analysis of trials in patients with NSTEACS showed that Hirudin reduced the incidence of death/MI by 20% during the treatment period ***(The Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002)***.

E- Oral anticoagulation (vit. K antagonists):

Vitamin K antagonists treatment in combination with aspirin was shown to be more effective than aspirin alone in the long-term prevention of death, recurrent MI and stroke ***(Hurlen et al., 2002)***, but at the cost of a higher risk of major bleeding ***(Andreotti et al., 2006)***.

5-Coronary revascularization:

Based on multiple randomized trials, an early invasive strategy is recommended in patients with NSTE-ACS with ST segment changes and/or positive troponin on admission or that evolves over the next 24 hours***(Mehta et al., 2005)***. There are two methods for revascularization PCI &CABG.

A- Percutaneous Coronary Intervention (PCI):

The goals of PCI in patients with NSTEMI are to relieve symptoms and to improve prognosis (e.g., prevent death, MI, and recurrent ischemia). Coronary stents reduce the the rate of abrupt vessel closure and restenosis after PCI ***(Thygesen et al., 2012).***

B- Coronary Artery Bypass Grafting (CABG):

CABG is an excellent treatment for relieving angina and grafts protect against proximal minor plaque instability. The decision to refer the patient

for CABG involves many factors including age, comorbidities, severity of coronary atherosclerotic disease ***(Thygesen et al., 2012).***

6-Statins:

The concept that lipid lowering may stabilize plaques is supported by numerous trials showing that statin therapy reduces the risk of clinical events despite only modest angiographic reductions in the severity of coronary stenosis ***(Brown et al., 1990)***.

CD154 (CD40 Ligand)

CD40L is a trimeric, transmembrane protein of the tumor necrosis factor family that was originally identified on cells of the immune system (activated CD4+ve cells, mast cells, basophils, eosinophils, and natural killer cells ***(Andre et al., 2002a)***.

The role ofCD40L in the immune response involves binding to its receptor on B cells, CD40, to induce B-cell proliferation, generate memory B cells, block B-cell apoptosis, and mediate antibody class switching ***(Andre et al., 2002a).***

However, it was subsequently shown that CD40L and CD40 are also present on several cellsof the vasculature, including endothelial cells, smooth muscle cells, monocytes, and macrophages. Additionally, it was found thatCD40L and CD40 also exist in platelets ***(Andre et al., 2002a).***

Much attention has been focused on CD40L's cryptic existence in platelets and its potential role in mediating a platelet-dependent inflammatory response associated with the atherothrombotic state. Several studies showed platelet-associated CD40L to elicit an inflammatory response from endothelial cells(ECs) and induce monocytic, endothelial and vascular

smooth muscle tissue factor expression in a CD40/CD40L-dependent manner ***(Anand et al., 2003).***

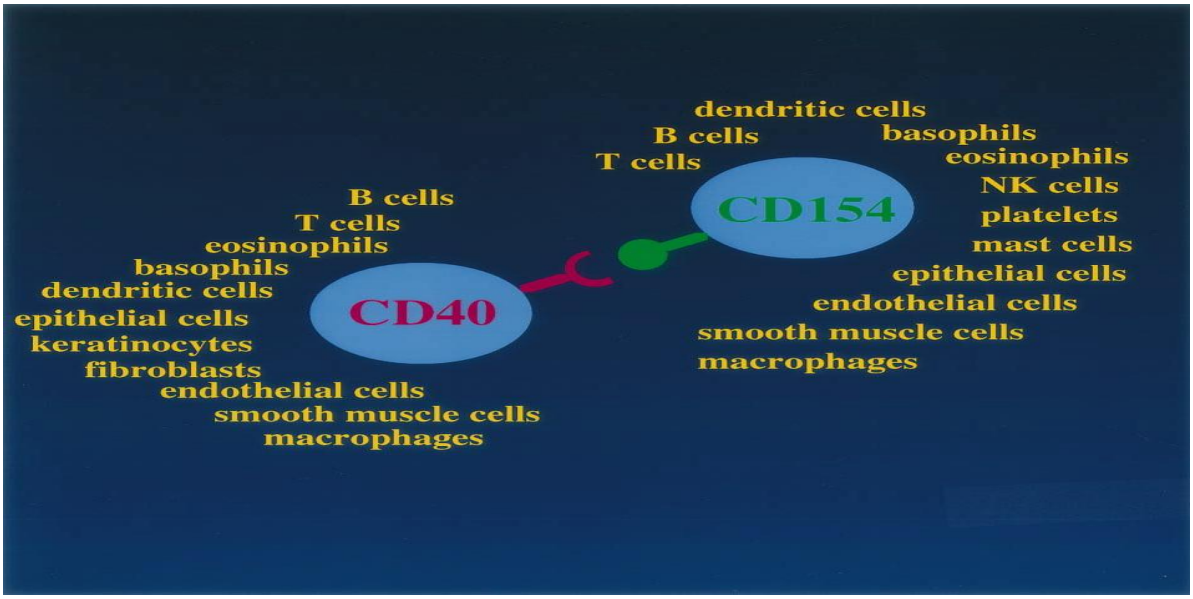

Figure 3. Human cell types expressing CD40 and CD40L (CD154) ***(Schonbeck and Libby, 2001)***

Structure of CD40L

Gene:

HumanCD40L cDNA was obtained by screening activated peripheral blood T lymphocytes with the respective murine probe ***(Armitage et al., 1992).***The 13-kbp DNA sequence for CD40Lshares 80% overall homology with its murine counterpart ***(Tsitsikov et al., 1994).***

Mapped to chromosome X, region q26.3-q27.1,the gene is composed of five exons and four intervening introns, and encodes a 2.3-kbp mRNA, which upon transcription yields a polypeptide consisting of 261amino acids (260 amino acids for the murine ligand) (fig.4).The large, 215 amino acid-long (214 amino acids for the murine ligand), cysteine-enriched (four cysteine residues) carboxy-terminal extracellular domain is mainly encoded by exons II-V, whereas the small transmembrane (24 amino acids) and amino-

terminal intracellular (22 amino acids) domains are encoded by exon 1***(Kotten and Banchereau, 1996).***

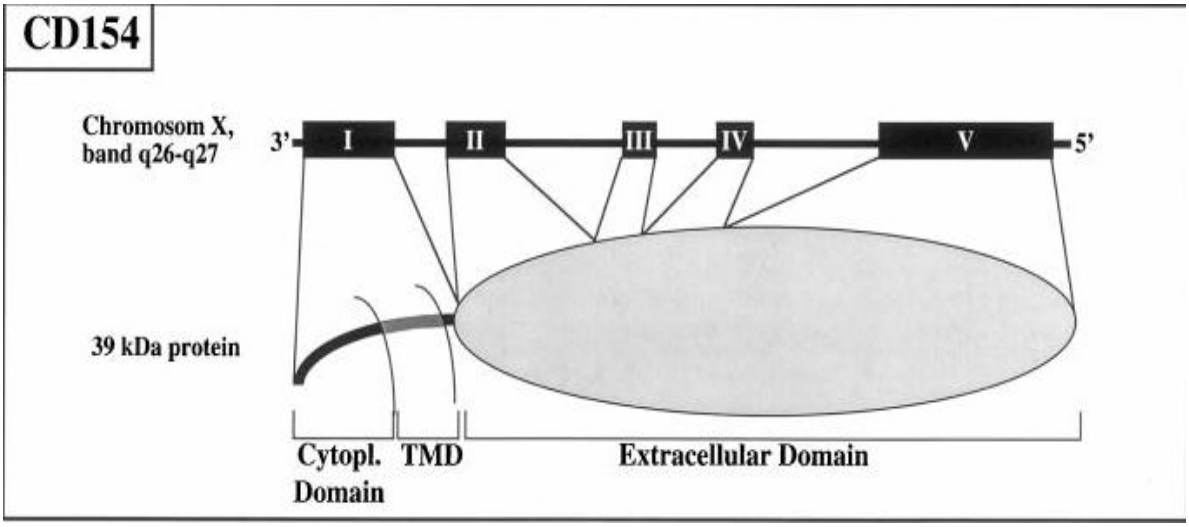

Figure (4): Organization of the human gene and protein for CD40L(CD154). Shown are the genomic (top) and protein (bottom) organization of CD40L. Exons are indicated as black boxes, labeled with the respective exon number ***(Schonbeck and Libby, 2001)***.

Protein:

Beside the cell-associated full-length 39-kDa protein, shorter soluble forms of the ligand have been described with a molecular weight of 31, 18, and 14 kDa. Then an additional 33-kDa CD40L species was reported in murine B cells ***(Wykes et al., 1998)***.

The 18-kDaform, which lacks the cytoplasmic tail, the transmembrane region, and parts of the extracellular domain, is functional and is considered the soluble form of this cytokine ***(Wykes et al., 1998)***.

Molecular structure:

Despite the structural homology of the CD40L receptor-binding domain to other TNF gene superfamily members, considerable differences exist in several loops, including those predicted to be involved in CD40 binding. In particular, neither the extracellular

domain of CD40L, which consists of a 75-amino acid located immediately adjacent to the membrane-spanning region, nor the receptor-binding domain, consisting of two overlying sheets are shared with other TNF gene superfamily members ***(Karpusas et al., 1995).***

The interaction between the receptor and its ligand is stabilized by charged residues, with CD40L presenting basic chains (K143, R203, R207) and CD40 presenting acidic side chains (D84, E114, E117). A wall of hydrophobic residues surrounds the polar interacting groups in the CD40L/CD40 complex ***(Singh et al., 1998)***.

Cell types expressing CD40L

The synthesis of CD40L was originally thought to be restricted to activated CD4+T lymphocytes, including cells of the Th0, Th1, and Th2 subtype. However, succeeding studies demonstrated that further T lymphocyte subpopulations as well as other leukocytic and non-leukocytic cell types express CD154 ***(Schonbeck and Libby, 2001)*****(Fig. 3).**

1. T lymphocytes. Apart from CD4+ T lymphocytes, further subpopulations are capable of expressing CD40L mRNA and/or protein, including CD8+, CD45RO+/CD45RA+, or Tc1/Tc2 T lymphocyte subsets, as well as CD4/ CD8-negative T lymphocytes ***(Sad et al., 1997***). Inducibility of CD40L expression on T lymphocytes seems to depend on maturation, since immature thymocytes do not express CD40L on their surface after stimulation, indicating acquisition of the ability to express the ligand late in thymocyte development ***(Fuleihan. et al., 1995).***

2. Basophils. Freshly isolated purified human peripheral blood basophils as well as the human basophilic cell line KU812 express (upon activation) functional CD40L, capable of inducing IgE production, suggesting that

basophils might play an important role during allergy, not only by producing inflammatory mediators, but also by directly regulating IgE production independently of T lymphocytes ***(Gauchat et al., 1993) and (Yanagihara et al., 1997).***

3. Eosinophils. Peripheral blood eosinophils as well as the eosinophilic cell line EOL-3 express functional CD40L upon activation, whereas eosinophils from hypereosinophilic patients express CD40L constitutively, indicating a role for CD40L in the inflammatory processes involving eosinophil infiltration and activation ***(Gauchat et al., 1995).***

4. Monocytes/macrophages and Kupffer cells. Initial observations in mononuclear phagocytes revealed CD40L mRNA within monocytes extracted from human peripheral blood ***(Cocks et al., 1993).***

Later studies demonstrated the inducibility of CD40L mRNA and biologically functional protein in human peripheral blood monocytes in vitro by cytokines, such as IL-1 or TNF-α, and enhanced expression of the ligand on activated monocytes in situ within human atherosclerotic lesions as well as during chronic allograft rejection in human liver allografts***(Afford et al., 1999.***

5. Natural killer cells. Natural killer (NK) cells contain CD40L transcripts and show enhanced CD40L expression upon stimulation with IL-2. The ligand is functional as demonstrated by the killing capability of CD40L-positive, but not CD40L-deficient, NK cells. These studies suggested a potential role for CD40L in NK cells in immune responses against B cell malignancies **(Carbone et al., 1997**).

6. B lymphocytes. Purified human peripheral blood B lymphocytes, as well as a variety of B lymphoblastoid cell lines and hybridomas can express functional CD40L following activation, which gave rise to the presumption that this mediator might facilitate responses of activated B lymphocytes

(Grammer et al., 1995) and (Clodi et al., 1998). It was also reported that malignant B lymphocytes coexpressed CD40 and CD40L protein ***(Blossom et al., 1997).***

7. Platelets. Thrombocytes were identified as another source of CD40L ***(Henn et al., 1998)***. Expression of the ligand was observed only seconds after activation of the platelets in vitro and in the process of thrombus formation in vivo. Further studies demonstrated the biological functionality of the ligand, since it induces expression of chemokines, adhesion molecules, and tissue factor, and diminishes the expression of thrombomodulin in human vascular endothelial cells ***(Slupsky et al., 1998).***

8. Mast cells. The human mast cell line HMC-1, freshly isolated purified human lung mast cells, as well as nasal mast cell from patients with perennial allergic rhinitis express functional CD40L in vitro and in situ ***(Pawankar et al., 1997) .***

9. Dendritic cells. Human blood dendritic cells express constitutive CD40L mRNA and protein. Interestingly, ligation of CD40 induces expression of the CD40L gene leading to a rise in ligand levels on the dendritic cell surface. Dendritic cell CD40L is functional, as demonstrated by the finding that CD40L-deprived dendritic cells lose their capability to regulate B cell activation and maturation ***(Pinchuk et al., 1996).***

10. Endothelial and smooth muscle cells. Human vascular endothelial and smooth muscle cells express functional CD40L in vitro and at sites of inflammation, e.g., atherosclerotic lesions as well as rejected cardiac and renal allograft transplants, in situ ***(Gaweco et al., 1999).***Cultured endothelial and smooth muscle cells express little constitutive CD40L, but show marked increases 12–24 h after stimulation with IL-1, TNF-α, IL-4, or IFN-γ **(*Mach et al., 1997 b)***.

11. Epithelial cells. CD40L expression is induced on glomerular and tubular epithelial cells during human chronic renal allograft rejection, but seems to be absent on normal human bronchial epithelial cells ***(Gaweco et al., 1999) and (Gormand et al., 1999)***.

Soluble CD40 Ligand (sCD40L)

CD40L is cryptic in unstimulated platelets but is rapidly presented to the platelet surface after platelet stimulation. The surface-expressedCD40L is subsequently cleaved over a period of minutes to hours, generating a soluble fragment termed sCD40L that remains trimeric. Studies on the cellular distribution of CD40L indicate that >95% of the circulating CD40Lexists in platelets. This suggests that platelet stimulatory events must be considered in the biological and pathological context of CD40L function (Figure 5) ***(Andre et al., 2002a).***

Several studies suggested that sCD40L is generated by intracellular proteolytic cleavage of the full-length form, producing an 18-kDa fragment starting at methionine 113 that lacks the trans-membrane as well as parts of the extracellular domain, but conserves the CD40 ligation domain ***(Graf et al., 1995).***

Consequently, sCD40L retains the ability to ligate CD40. Although the proteolytic activity implicated in the formation of sCD40L involves mammalian adamalysins (ADAMs), which belong to the group of metalloproteinases, the analogy of sCD40L to TNF-α might suggest proteolytic pathways, resembling the function reported for the TNF-α converting enzyme (TACE) ***(Black et al., 1997) and (Black and White, 1998).***

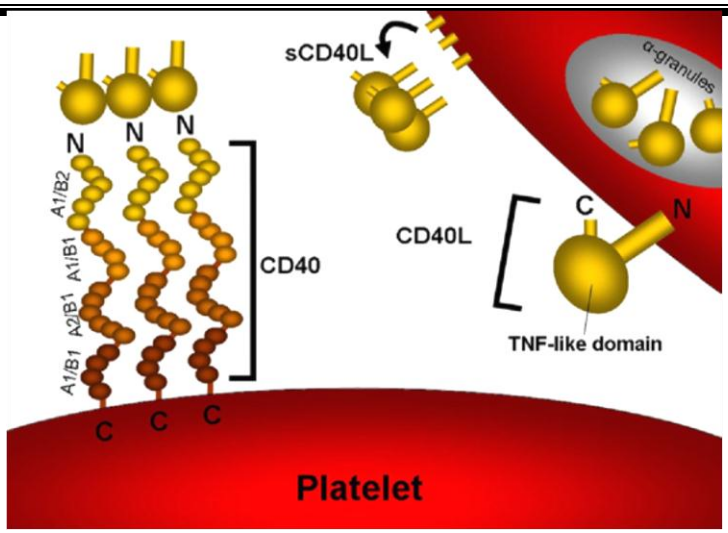

Figure 5. Structure of CD40 Receptor, CD40L, and sCD40L ***(Antoniades et al., 2009).***

Regulation of soluble CD40L expression

Platelets express CD40L after stimulation with a wide range of platelet activators, such as thrombin and thrombin receptor agonists, for example, collagen, phorbol myristate acetate, and so forth. The first studies reported that CD40L expression on platelet surface is dependent on intracellular calcium (Ca2+) concentrations and protein kinase C activation ***(Antoniades et al., 2009).***

Elevated release of soluble CD40ligand (sCD40L) has also been observed in platelets from diabetic patients after stimulation with thrombin or thrombin receptor activation peptide. It was also found that insulin resistance as well as glucose and advanced glycation end products induce sCD40L release from platelets and increase CD40Lexpression in murine megakaryocytes ***(Varo et al., 2005).***

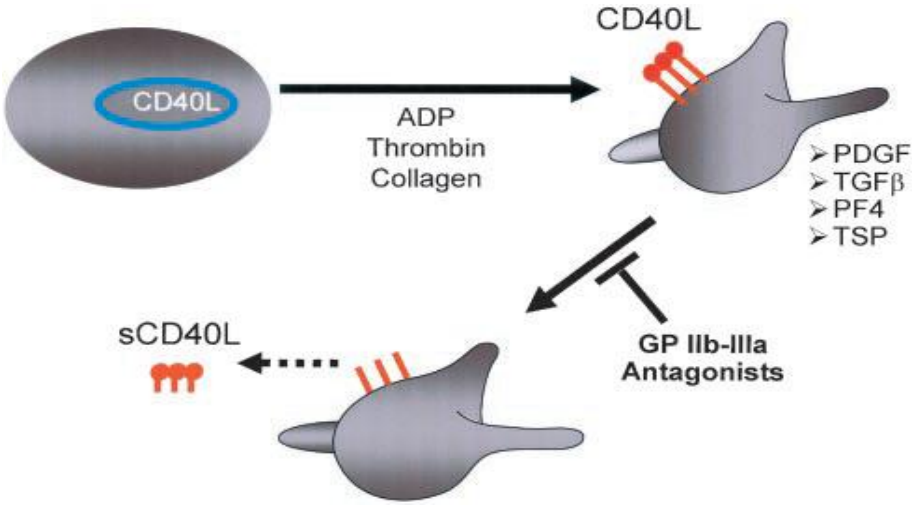

Figure 6. The shedding of soluble sCD40L during platelet stimulation ***(Andre et al., 2002a***).

The classical CD40L receptor, CD40

CD40, the classical CD40L receptor, was initially identified as a molecule expressed on B cells, at all stages of development and differentiation. Later, CD40 expression was shown to be exhibited by monocytes, macrophages and dendritic cells. Further studies identified this TNFR (Tumor necrosis factor receptor) family member on other cell types, including non-hematopoetic cells ***(Schonbeck and Libby, 2001)*** **.**Fig(3)

In the vasculature, CD40 was shown to be expressed on ECs (Endothelial cells), SMCs (Smooth muscle cells), macrophages and platelets. The CD40 molecule was shown to be expressed as a monomer. Upon its oligomerization by binding to its ligand, CD40L, or to crosslinking antibodies (anti-CD40), dimerization of the receptor was triggered (Fig. 7). Such dimer formation, exhibited between cysteine residues located at position 238 in the intracellular region of CD40, was shown to be essential for some CD40 signaling events. These CD40-mediated signals included phosphoinositide-3kinase (PI-3K) activation and the subsequent B7-2 upregulation, as well as the production of IL-8 (Fig. 7) ***(Reyes-Moreno et al., 2007).***

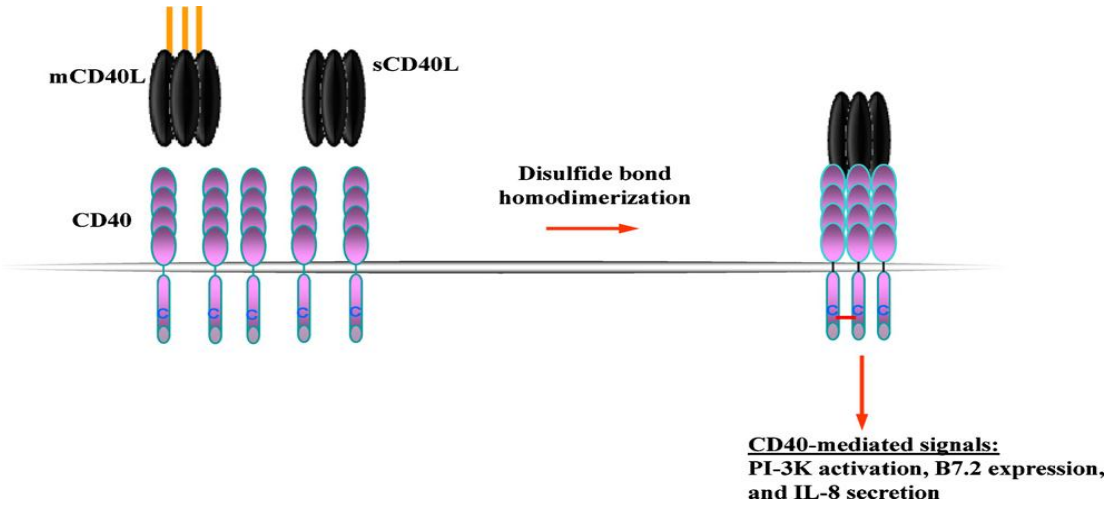

Fig. 7. Binding of Membrane-bound or soluble CD40L to CD40 ***(Hassan et al., 2012)***.

Structure of CD40

Gene:

The CD40 gene encodes a single1.5-kbp mRNA species and maps to human chromosome20, bands q12‑q13.2 ***(Lafage-Pochitaloff et al., 1994).***

Transcription of the gene results in a 277-amino acid membrane-bound protein that consists of a 22- amino acid signal sequence, a 171-amino acid extracellular domain, a single 22-amino acid transmembrane domain, and a 62-amino acid cytoplasmic domain (fig. 8) ***(Hostager et al., 1996).***

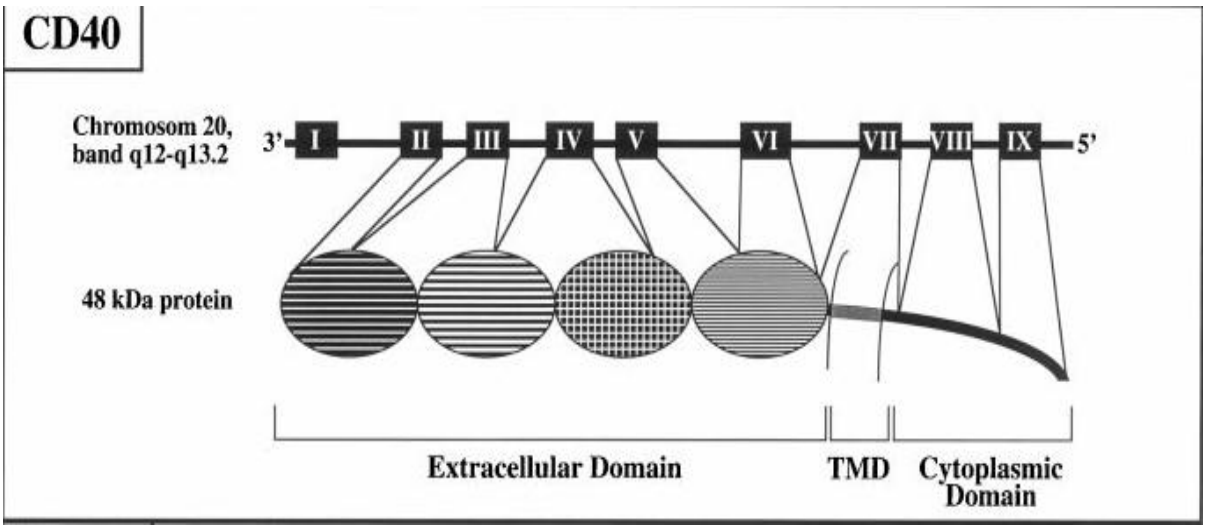

Figure 8. Organization of the human gene and protein for CD40. Shown are the genomic (top) and protein (bottom) organization of CD40. Exons are indicated as black boxes, labeled with the respective exon number ***(Schonbeck and Libby, 2001)***.

Protein:

Translation of the 1.5-kbp CD40 mRNA generates animmunoreactive protein with a molecular weight of43‑50 kDa, mostly reported as a doublet consisting of a43-kDa and a47-kDa protein. Furthermore, dimer formation has been described in B lymphocytes ***(Schonbeck and Libby, 2001)***.

As typical for members of the TNF receptor superfamily, CD40 is characterized by a repetitive amino acid sequence pattern of four cysteine-enriched subdomains, typically consisting of six cysteines forming three disulfide domains. The intracellular domain of CD40, however, does not display a close relationship to other members of the family. The cytoplasmic domain of CD40 contains at least two major signaling determinants that include threonine 227 and 234 ***(Hostager et al., 1996).***

Cells expressing CD40

1. Expression of CD40 in ECs:

In the vessel, the endothelium functions in transmitting signals from the circulating blood to tissues in the vessel wall. In doing so, ECs put in action a number of membrane-bound as well as intracellular molecules. CD40 molecules have been identified on ECs, and implicated in a number of interactions leading to cellular response. In fact, vascular ECs have been shown to express CD40in a number of tissues and vascular beds. Immunohistochemical staining of frozen sections from normal spleen, thyroid, skin, muscle, kidney, lung, and umbilical cord, showed CD40 expression in vascular ECs ***(Schonbeck et al., 1997a).***

Such constitutive expression ofCD40 was confirmed by ***Vowinkel et al.,2006***, who reported significant presence of CD40 molecule in several vascular beds, with lung, kidney and small intestine exhibiting the highest expression,whereas liver and stomach showed no detectable baseline levels.

Mach et al., 1997b reported a basal CD40 expression by ECs in normal human arteries. They also found little expression of CD40in saphenous vein ECs, in situ.

2. Expression of CD40 in SMCs

In the vasculature, CD40/CD40L dyad is also expressed in SMCs. Indeed, SMCs derived from saphenous veins were shown to exhibitCD40 molecules constitutively on their surface ***(Mach et al., 1997b) and (Schonbeck et al., 2000***).

Moreover, stimulating cells of saphenous vein or aortic origin, with IL-1β, TNF-α, or IFN-γ increased surface levels and de novo synthesis of CD40. However, in situ CD40 expression has not been reported in SMC of normal arteries, even though itwas well exhibited in cells of atherosclerotic artery segments ***(Mukundan et al., 2004).***

3. Expression of CD40 in macrophages

Other vascular cells expressing CD40 are macrophages. Indeed, CD40 was shown to be constitutively expressed at both, the mRNAand the protein level, in cultured vascular macrophages and inmonocyte-derived macrophages originating from blood of healthy donors. Such expression was upregulated by IFN-γ ***(Mulhaupt et al., 2003).***

4. Expression of CD40 in platelets

Platelets have been shown to express CD40 ***(Danese and Fiocchi, 2005).***Such CD40 was shown to be implicated in platelet activation, as discussed below. It is worth noting here that expressing both CD40L and CD40 rendered platelets important contributors to inflammation as the CD40L/CD40 interaction would allow a crosstalk between platelets and vascular cells such as ECs, and blood leucocytes ***(Geraldes et al., 2006).***

Table3. Expression of CD40 and CD40L in vascular or immune Cells ***(Rizvi et al., 2008)***

Cells	CD40	CD40L
Platelets	+	+
Monocytes and macrophages	+	+
Endothelial cells	+	+
Neutrophils	+	-
Smooth muscle cells	+	-
Mast cells	+	+
Fibroblasts	+	-
T cells	+	+
B cells	+	-
Dendritic cells	+	-

CD40–CD40L signaling pathways

In platelets, sCD40L induces β3 integrin tyrosine phosphorylation and triggers platelet activation by outside-insignaling ***(Prasad et al., 2003).***

CD40-mediated signaling in monocytic cells upregulates TNF receptor-associated factor (TRAF) mRNAs (messenger ribonucleic acid) and MAPK (Mitogen-activated protein kinase) signaling pathways ***(Pearson et al., 2001)***. In CD40LinducedDC (denderitic cell) activation, the roles of p38 MAPK, ERK (Extracellular signal-regulated kinases) andPI3-kinase (Phosphatidylinositide 3 Kinase) signal transduction pathways and the expansion of virus-specific CD8+ve T cell memory responses have been reported ***(Yu et al., 2004).***

Conversely, after CD40 ligation in carcinoma cells, a proapoptotic response occurs after inhibition of PI3-kinase, ERK and MAPK-regulated protein synthesis ***(Davies et al., 2004)***.

CD40-induced ROS (reactive oxygen species) production by NADPH (reduced form of Nicotinamide adenine dinucleotide phosphate) oxidase in WEHI 231 cells (a murine immature B lymphoma cell line) requires TRAF3, as well as the activities of PI3-kinase and Rac1 (Ras-related C3 botulinum toxin substrate 1) ***(Ha and Lee, 2004).*** CD40L inhibits EC migration by increasing production of endothelial ROS ***(Urbich et al., 2002).***

Moreover, CD40-dependent activation of thePI3-kinase/Akt pathway mediates EC survival and in vitro angiogenesis ***(Deregibus et al., 2003).*** Oxidative stress promotes blood cell–endothelial interactions in the microcirculation ***(Cooper et al., 2002).***

Upregulation of CD40, CD40L and platelet-monocyte aggregates in cigarette smokers indicate a CD40–CD40Lassociation with enhanced oxidative stress ***(Harding et al., 2004).***

It has been shown that recombinantCD40L induces ROS generation in platelets and ECs ***(Chakrabarti et al., 2005)and (Chakrabarti et al., 2007).*** CD40 ligation in platelets also leads to activation of p38 MAPK , Akt and enhanced oxidative stress, leading to reactive oxygen intermediate generation ***(Chakrabarti et al., 2005) and (Danese et al., 2004)***.

Other CD40 ligand Receptors

1. The αIIbβ3 integrin (Glycoprotein IIb/IIIa)

The αIIbβ3 integrin, exclusively expressed on platelets and megakaryocytes, is a member of the integrin superfamily that binds to molecules such as fibrinogen and vitronectin, containing the RGD (Arginine-Glycine-Aspartate)sequence. By interacting with its natural ligands, αIIbβ3 is directly implicated in platelet function and thrombosis. In 2002, this integrin was identified as a receptor for CD40L **(Fig. 9)** ***(Andre et al., 2002a) and (Prasad et al., 2003).***

Indeed, it was shown that recombinant sCD40L was capable of binding to αIIbβ3 integrin on activated platelets and therefore inducing platelet spreading, promoting platelet aggregation under high shear rates, as well as allowing stability of arterial thrombi **(*Andre et al., 2002b).***

Prasad et al. (2003) further reported that CD40L is a primary platelet agonist capable of inducing platelet activation (induction of fibrinogen binding and the formation of platelet microparticles), by binding to its αIIbβ3 receptor and triggering outside-in signaling**.** In addition, engagement of αIIbβ3 by CD40L or other ligands, inducing platelet adhesion, was shown to upregulate CD40L surface exposure on platelets, enhancing as such the interaction of platelets with CD40+ cells, including ECs ***(May et al., 2002).***

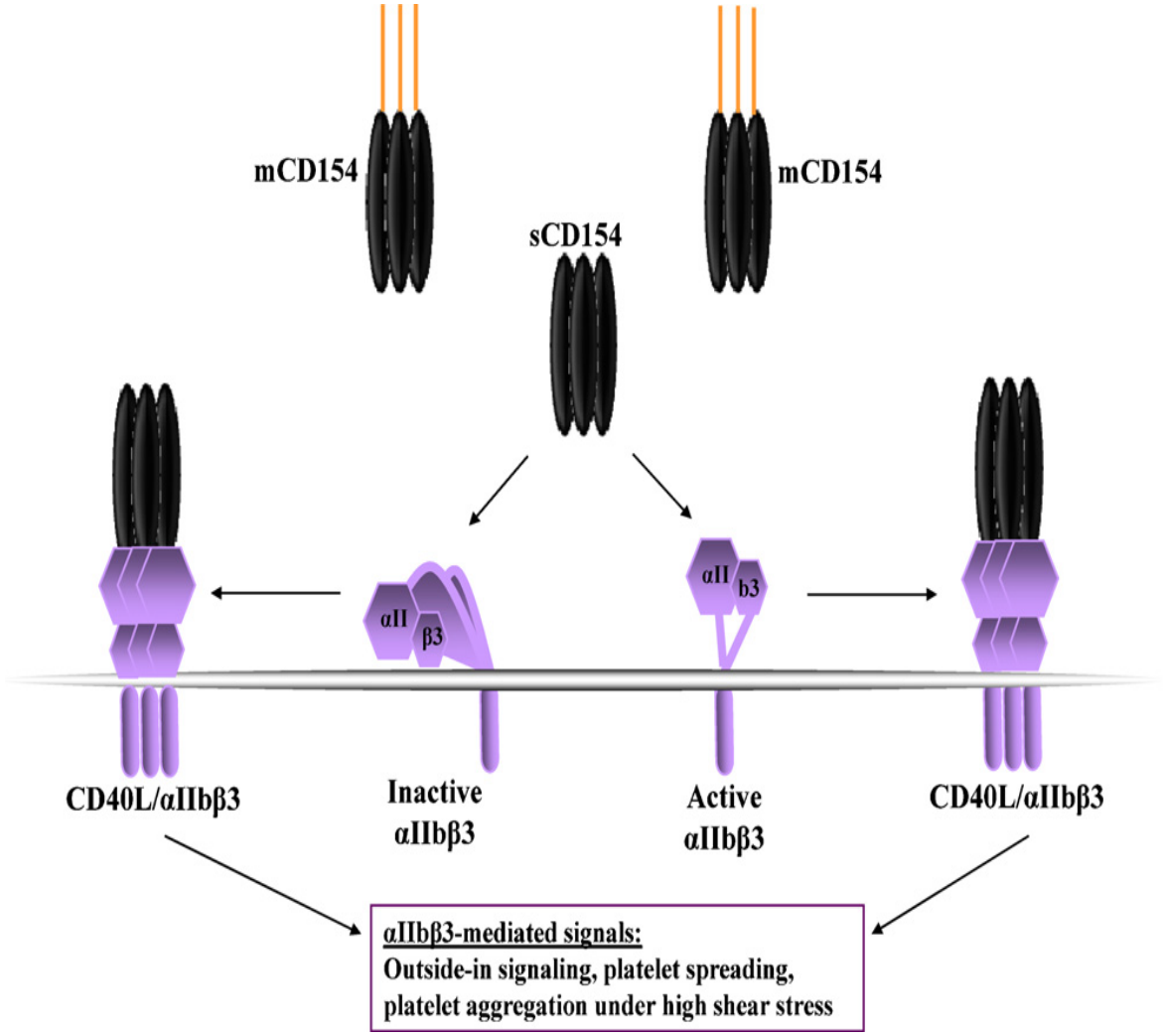

Fig.9. Binding of CD40L to αIIbβ3 integrin: Membrane-bound and soluble CD40L, in their trimeric form can bind to αIIbβ3 integrin. Such interaction implicates both active and inactive forms of the αIIbβ3 integrin (i.e., on activated and resting platelets, respectively)***(Hassan et al., 2012).***

2. The α5β1 integrin

The α5β1 integrin also binds to fibrinogen and vitronectin via their RGD sequence. It is implicated in cell adhesion, migration, proliferation as well as survival of many cell types. Interestingly, it was shown that the α5β1 integrin also serves as a relevant receptor for CD40L **(Fig. 10).** Binding of CD40L to cells expressingα5β1 integrin leads to the phosphorylation of the extracellular signal regulated kinases 1/2 (ERK 1/2) and expression of IL-8 mRNA in these cells. However, unlike the natural ligands of α5β1,

fibrinogen and vitronectin, CD40L binds to the inactive rather than the active form of α5β1 **(Fig.10).** Interestingly, CD40L/α5β1 interactions did not interfere with the binding of CD40L to CD40, indicating the capacity of CD40L to bind simultaneously to both receptors***(Leveille et al., 2007).***

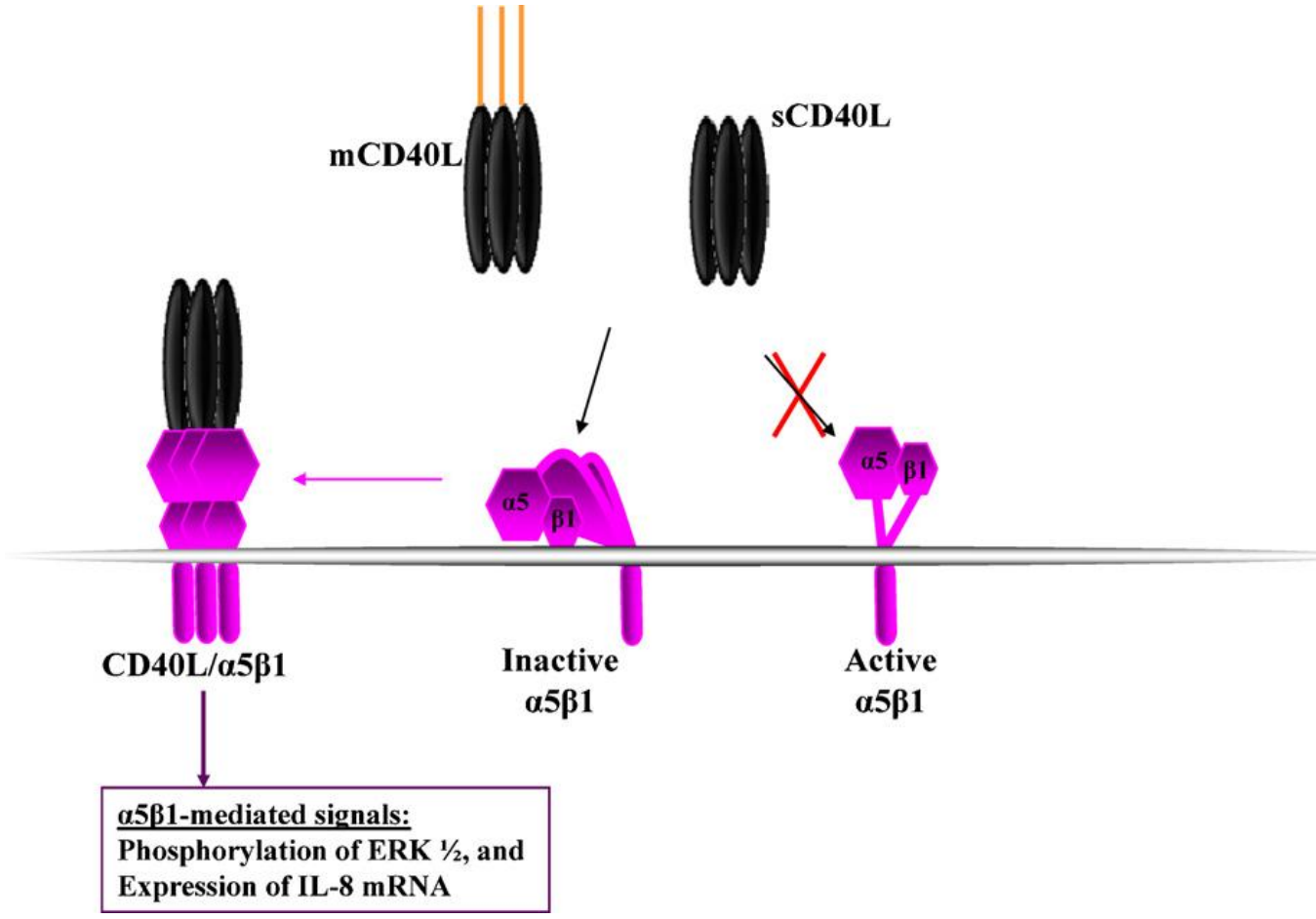

Fig.10. Binding of CD40L to the α5β1 integrin: Trimeric form of membrane-bound and soluble CD40L is capable of binding to the newly identified CD40L receptor, the α5β1integrin. Such binding, unlike that of other α5β1 ligands, necessitate the inactive rather than the active form of the integrin ***(Hassan et al., 2012).***

The α5β1 integrin in ECs

This integrin plays a crucial role in EC biology. Indeed, α5β1 was found to support growth of ECs. Being a receptor for fibrinogen, α5β1 was implicated in attachment, spreading, migration as well as proliferation of ECs in the extracellular matrix ***(Breithaupt-Faloppa et al., 2006) and (Wang and Milner, 2006)***.

These phenomena are essential for the process of angiogenesis, where ECs would produce extracellular matrix on which they will attach, spread, migrate, and proliferate in order to form new vessels. Indeed, α5β1was found to be essential for angiogenesis mediated by various vasoactive substances including angiotensin-II (Ang II), and vascular-endothelial growth factor-receptor-1(VEGFR-1) ***(Cascone et al., 2005).***

The α5β1 integrin in SMCs

In these cells, α5β1 was also found to be implicated in cell adherence and migration onto extracellular matrix. In fact, SMCs migrate from the media to the intima layer of the vessel wall in response to various substances including cytokines such as IL-1β, TNF-α, and IFN-γ possibly released by activated immune cells that infiltrated the vessel wall, and acting on the α5β1 to upregulate its expression ***(Barillari et al., 2001)***.

These phenomena under lie many pathological conditions of the vascular system. Moreover, α5β1 in SMCs was found to potentiateCa2+ channels in SMCs, and therefore playing a role in contractility of these cells and their function in blood vessel homeostasis ***(Gui et al., 2006).***

The α5β1 integrin in macrophages

The α5β1 integrin has been identified as a receptor for CD40L in a human monocyte/macrophage cell line, the U937 cells. As mentioned above, sCD40L was found to exhibit a biologically active binding to U937 cells via the α5β1 integrin ***(Leveille et al., 2007).***

The natural α5β1 ligands, namely fibrinogen, mediate the role of the integrin in phagocytic functions and cell adhesion of tissue macrophages, processes important in host defense and wound repair ***(Blystone et al., 1994).***

In vascular macrophages, increased cell adhesiveness to fibronectin via several integrins, including theα5β1, is important in the tethering of cells in inflamed tissues such as the atherosclerotic lesion site ***(Seales et al., 2005).***

The α5β1 integrin in platelets

In the vasculature, α5β1 is also expressed on platelets. In addition to the major platelet receptor for fibrinogen, αIIbβ3, platelet α5β1 represent another link between platelets and fibrinogen fibrils, allowing platelet adhesion to fibrinogen surfaces ***(Rahman et al., 1998).***

Both integrins switch from a quiescent state, unable to bind to fibrinogen/fibrin, to an active state, able to bind strongly to these extracellular matrix proteins, via an RGD motif-dependent pathway***(Hassan et al., 2012).***

Another common ligand for both αIIbβ3 and α5β1 integrins, is the CD40L. Even though, CD40L/α5β1 interactions have not been documented in platelets, the α5β1 integrin could be another potential player inCD40L-induced activation/aggregation functions, and therefore in platelets-associated events such as thrombosis. This possibility is currently under investigation, using in vitro as well as in vivo techniques ***(Hassan et al., 2012).***

3.The αMβ2 integrin

Also known as Mac-1, αMβ2 is mainly expressed on neutrophils as well as monocytes/macrophages. This integrin has been recently identified as a receptor for CD40L (Fig. 11) ***(Sandilands et al., 2006) and (Zirlik et al., 2007).***

Indeed, the binding of soluble CD40L to phorbo-12-myristate-13-acetate (PMA)-activated monocytes cells as well as to Chinese hamster ovary cells

tranfected with the active form of Mac-1, was inhibited by anti-Mac-1 antibodies. In addition, CD40L/Mac-1 interactions were capable of stimulating adhesion and migration of monocytes as well as their myeloperoxidase release in vitro. Studies further emphasized that interaction of CD40L with Mac-1 and not with CD40, elicited adhesion between ECs and monocytes at the site of plaque formation, producing necessary cytokines, chemokines and myeloperoxidases and leading to the development of inflammation and atherosclerosis ***(Zirlik et al., 2007).***

Later on, CD40L was even shown to upregulate the expression of Mac-1 on neutrophils, mediating as such their cross talk with activated platelets at the site of injury. However, more insights are needed as to the implication of CD40L/Mac-1 crosstalk in the pathophysiology of the vascular system ***(Li et al., 2008)***.

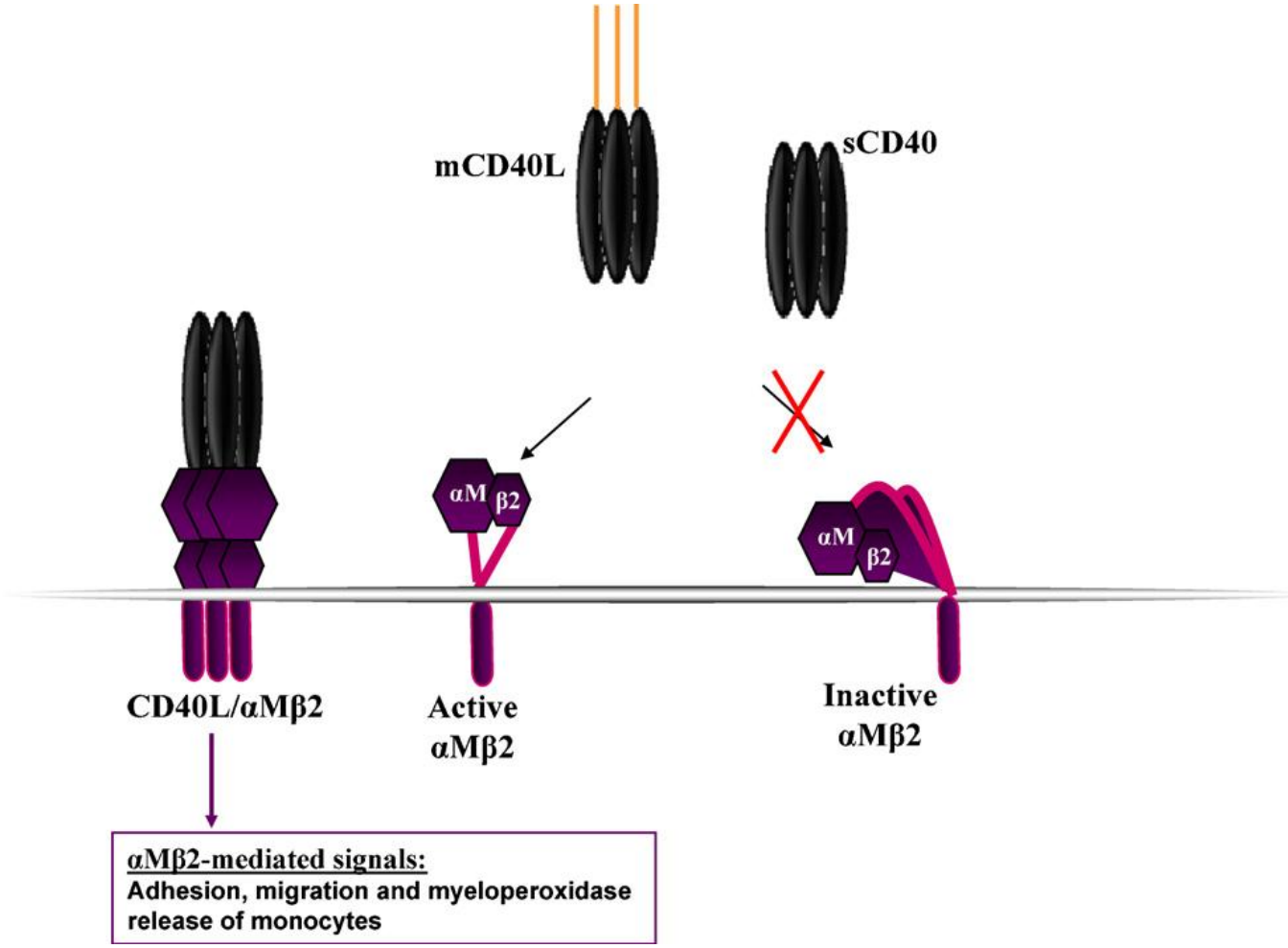

Fig.11. Binding of CD40L to Mac-1 (the αMβ2 integrin): Trimeric membrane-bound and soluble CD40L, bind to another newly identified receptor, the Mac-1 integrin, in its active form ***(Hassan et al., 2012).***

Role of sCD40 Ligand in ACS Pathogenesis

The rupture of an atherosclerotic plaque or endothelial erosion is the underlying cause of arterial occlusion, frequently leading to athero-thrombosis-associated manifestations including myocardial infarction, unstable angina, stroke, or even sudden death ***(De Caterina et al., 2010).***

Indeed, increased levels of CD40L have been found in patients with asymptomatic hypercholesterolemia, unstable angina, and acute myocardial infarction. Studies have shown that soluble and T cells- and platelets-membrane-bound CD40L were upregulated in patients with angina, with particularly high levels in patients with unstable angina ***(Aukrust et al., 1999).***

Similarly, in another study, increased levels of sCD40L were observed in patients with unstable angina, and more importantly in patients with acute myocardial infarction ***(Garlichs et al., 2001a).*** In addition, the same group also reported a correlation between the CD40L system and hypercholesterolemia, a major risk factor of atherosclerosis. Indeed, the expression of a biologically active CD40L on platelets and that of its receptor, CD40, on monocytes was shown to be increased in patients with moderate hypercholesterolemia compared to healthy subjects ***(Garlichs et al. 2001b).***

Taken together, these findings suggest that CD40L contribute to atherosclerosis, from initiation events to severe clinical manifestations of the disease ***(Hassan et al., 2012).***

CD40L axis in atherosclerosis

Research over the years has investigated the implication of several molecules and cellular processes in the etiology of vascular diseases in general and atherosclerosis in particular. In this context, CD40L/CD40 interaction was shown to be implicated at all stages of the atherosclerosis process, from initiation to disease complications. The expression of CD40 and CD40L has been demonstrated on macrophages, ECs, T lymphocytes, and SMCs in such lesions, and more intensely in macrophage- and T-cell-rich areas .The expression of CD40L as well as of its receptors seems therefore to correlate with atherosclerotic lesions, at different stages of the disease ***(Hakkinen et al., 2000).***

Initiation events

1. Biological role of CD40L in vascular ECs.

At the initial steps of atherosclerosis, ECs activated by oxidized low density lipoprotein (oxLDL) exhibit an upregulation of CD40L and CD40 mRNA and protein expression, mediated by activation of protein kinase C-α (PKCα) ***(Li et al., 2003).***

In addition, various cytokines are released from activated monocytes and T cells at the site of injury. These cytokines, namely TNF-α, IL-1, IL-3, IL-4, IFN-γ, or IFN-β induce an increased CD40 expression in ECs ***(Karmann et al., 1995).***

Such cytokine-mediated upregulation of CD40 was shown to implicate transcription factors such as nuclear factor kappa B (NF-ƙB), signal transducers and activators of transcription-1 (STAT-1), Janus activated kinases-1 and -2(JAK-1 and -2), and interferon regulating factor-1 (IRF-1)

(Geraldes et al., 2006). CD40+ve ECs are stimulated by CD40L derived from platelets or activated T cells. The biological effects of CD40L on ECs are summarized in **Fig. 12.**

These CD40L-stimulated ECs further release another supply of cytokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1) involved in recruiting leukocytes ***(Lievens et al, 2010).*** Secretion of MCP-3, macrophage inflammatory protein-3α (MIP-3α), IL-6 and IL-15 is also induced in ECs upon their stimulation with CD40L, contributing as such to many inflammatory processes at the injury site ***(Lutgens et al., 2007) and (Pluvinet et al., 2008)***.

In addition, CD40L induce other atherogenic effects in ECs, such as enhancing their production of the proatherogenic factor endothelin 1 (ET-1), and downregulating that of the vasodilating cardio-protective factor, apelin ***(Phipps, 2008) and (Pluvinet et al., 2008).***

Moreover, CD40L was demonstrated to decrease mRNA and protein expression of endothelial nitric oxide synthase (NOS) as well as the stability and activity of the enzyme, and to increase superoxide anion (O^{2-}) production, adding therefore to the proinflammatory environment at the lesion site ***(Chen et al., 2008).***

In addition, stimulation of ECs via CD40 induces the expression of adhesion molecules namely, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) allowing circulating monocytes and T cells to adhere to and transmigrate across the endothelial layer***(Kotowicz et al., 2000).***

An interesting finding at this level is the role of CD40L in mediating the formation of platelet–leukocyte aggregates, facilitating the recruitment of leukocytes to the endothelial layer ***(Lievens et al., 2010).***

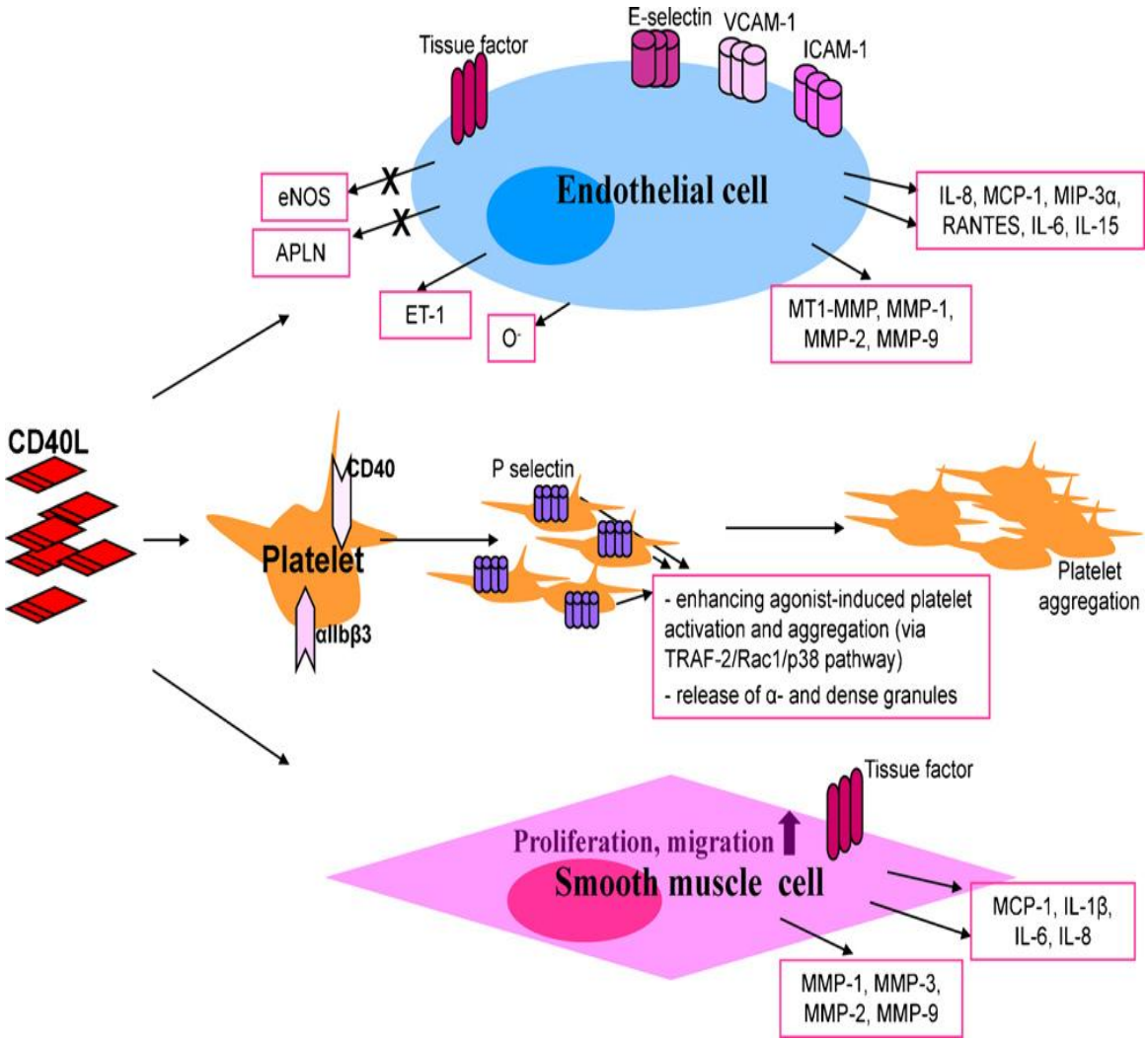

Fig.12. Role of CD40L interactions in ECs, SMCs, and platelets: CD40L induces many biological roles in vascular cells. ECs stimulated by CD40L were shown to exhibit an increased expression of their adhesion molecules (E-selectin, VCAM-1, and ICAM-1), and tissue factor, an increased production of proinflammatory chemokines (IL-8, MCP-1, MIP-3α, RANTES, IL-6, and IL-15), metalloproteinases (MT1-MMP, MMP-1, MMP-2 and MMP-9), and vasoactive factors (endothelin-1 (ET-1), superoxide anion (O_2^-)), and a downregulation of cardio-protective factors (apelin (APLN), endothelial nitric oxide synthase (eNOS)). In platelets, CD40L was shown to interact with at least two receptors, namely CD40 and αIIbβ3 integrin. By binding to the αIIbβ3 integrin, CD40L was shown to induce platelet spreading and activation. Other studies reported CD40L interacting with platelet CD40 capable of inducing P-selectin expression and platelet activation, as well as the release of their α- and dense granules, leading to platelet aggregation, while other groups demonstrated an enhancing effect of CD40L rather than an activating one in platelets. In SMCs, CD40L was shown to induce cell proliferation and migration, the production of proinflammatory chemokines (MCP-1, IL-1β, IL-6, and IL-8), and metalloproteinases (MMP-1,

MMP-3, MMP-2, and MMP-9), and to upregulate tissue factor expression***(Hassan et al., 2012).***

2. Biological role of CD40L in subendothelial macrophages and immunecells:

In the subendothelial lesion, CD40L interacting with its receptor(s) would induce more atherogenic responses in various cell types. Indeed, stimulation of CD40+ macrophages allows the release of several cytokines including IL-1β, IL-6, IL-12 and TNF-α**.** In addition, CD40L induces macrophages to secrete matrix metalloproteinases (MMPs) contributing to lesion instability and rupture ***(Lutgens and Daemen, 2002)***.

More recently, stimulation of macrophages via CD40 was shown to upregulate IL-12, inducible NOS, and MCP-1, and to decrease the anti-inflammatory cytokine IL-10, conferring to macrophages a pro-inflammatory rather than a wound-healing phenotype ***(Lutgens et al., 2010).***

Furthermore, the CD40L/CD40 dyad which is capable of inducing a bidirectional signaling, stimulates IL-12 release from activated T cells ***(Reyes-Moreno et al. 2007).*** Interestingly, platelet CD40L was recently shown to induce a decrease in regulatory T cell population, generally know to be athero-protective, in favour of the pro-atherogenic effector T cell population ***(Lievens et al., 2010).***

It is worth noting here, that the interaction of CD40L, not only with CD40, but also with other CD40L receptors, in inflammatory, immune, and endothelial cells, was shown to contribute to transplant and graft rejection, a finding proved essential in designing anti-rejection therapies ***(Kirk et al., 2009).*** On the other hand, it was demonstrated that there is an anti-inflammatory function for CD40 in macrophages and dendritic cells. Such function was shown to depend on CD40 expression levels, and to enhance

the production of the anti-inflammatory cytokine IL-10, rather than proinflammatory factors ***(Mathur et al., 2004).***

5. Biological role of CD40L in vascular SMCs:

Soluble CD40L or membrane-bound CD40L upregulated in the subendothelial space also target SMCs in the vessel wall. Biological effects of CD40L in vascular SMCs are presented in Fig. 12. Indeed, sCD40L was shown to induce proliferation and migration of SMCs, enhancing therefore hyperplasia and neo-intima formation at the lesion site. Interestingly, sCD40L enhances these phenomena in SMCs by upregulating their MMP-9 mRNA and protein levels ***(Chai et al., 2009).***

Indeed, the increased MMP expression in CD40L-stimulated SMCs has been reported in many studies. In addition to MMPs, CD40L was shown to stimulate SMCs to secrete cytokines such as MCP-1, IL-1β, IL-6, and IL-8, contributing further to lesion progression and subsequent instability and rupture ***(Mukundan et al., 2004***).

It is worth noting at this point that an increased CD40expression as well as activity has been reported in SMCs treated with the proatherogenic factors, Ang II and ET-1, via reactive oxygen species (ROS) and NF-ƙB pathways, respectively, emphasizing as such the important role of CD40L/CD40 dyad in atherosclerosis, as well as the synergy between various vasoactive factors and molecules in disease development ***(Souzaet al., 2009) and (Browatzki et al., 2007).***

The atherogenic function of CD40L/CD40 dyad could even extend to the myofibroblast population in the atherosclerotic lesion. CD40L was demonstrated to induce cell proliferation, adhesion molecule expression, as well as proinflammatory cytokine production in fibroblasts, promoting as such aggravation of the atherosclerotic lesion ***(Xu et al., 2007).***

Such lesion, now termed fibrous plaque is formed of extracellular matrix proteins, infiltrating leukocytes, macrophages-derived foam cells, proliferating SMCs, and may be also myofibroblasts ***(Lutgens and Daemen, 2002).***

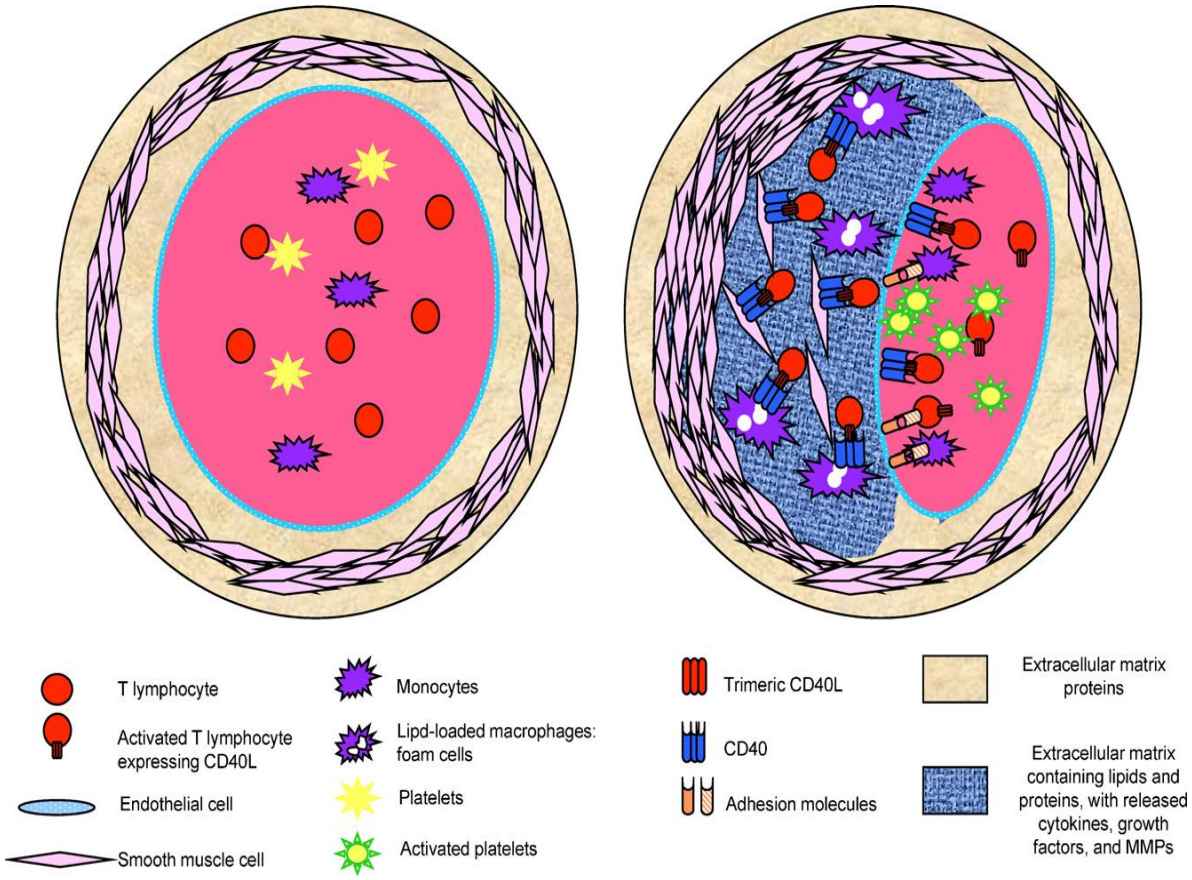

Fig 13.CD40L in atherosclerotic lesions: CD40L was shown to be implicated at different stages of atherosclerosis, from initial steps to advanced rupture-prone lesions. Initially, CD40L expressed on activated T cells would interact with CD40 expressed on activated endothelial cells inducing cytokine release a well as adhesion molecules expression, enhancing as such leukocyte infiltration into subendothelial space. Membrane-bound and soluble CD40L from infiltrated T cells would interact with CD40 on macrophages in the lesion (including foam cells), and on proliferating SMCs that migrated from the media to the intima layer. Such interactions would induce further cytokine release as well as production of MMPs rendering the lesions unstable and prone to being ruptured. At this point, lesions are rich with thrombotic components such as tissue factor which will activate the extrinsic coagulation cascade and CD40L itself which will activate

platelets. If these advanced lesions are ruptured, an atherothrombotic event is triggered ***(Hassan et al., 2012).***

Formation of rupture-prone lesion

An unstable fibrous plaque is characterized by an increased lipid content, increased inflammatory cells population, and a rather decreased extracellular protein deposition and smooth muscle population. Important players in this phenomenon are the MMPs, and the lesion neo-vascularization process ***(Hassan et al., 2012).***

A- MMPs inducing lesion rupture.

Various cell types in the lesion have been shown to upregulate their production of MMPs upon their stimulation with CD40L. CD40L-stimulatedmacrophages exhibit an increased MMP expression ***(Packard and Libby, 2008).***

In addition, CD40L induces MMP production in SMCs. Indeed, in vitro studies have demonstrated that human SMCs upregulated their synthesis and expression of MMPs, namely, MMP-1, MMP-3 and MMP-9 as well as the activation of MMP-2 upon their stimulation with CD40L ***(Schonbecket al., 1997b).***

Moreover, the MMPs-inducing function of CD40L targets also ECs. In this matter, CD40L expressed on activated platelets stimulated the production of MMPs, including MMP-1 and membrane type 1 MMP (MT1-MMP) and enhanced the activity of MMP-2and MMP-9 in ECs ***(May et al., 2002).***

In addition to the in vitro studies described above, the role of CD40L/CD40 dyad in MMP production and its importance in atherosclerosis has been also evidenced in situ. Indeed, atherosclerotic lesions in arteries

from animal models or human patients revealed an increased, and sometimes colocalized expression of CD40L/CD40 and MMPs ***(Wu and Li, 2006).***

B- Neo-vascularization contributing to lesion rupture.

In the process of lesion growth, an important aspect emerges being the formation of new vessels or vasa-vasora to ensure initially the oxygen and nutritional supplementation to the residing cells. This process, known as angiogenesis, was reported to implicate the CD40L/CD40 dyad. Indeed, CD40L was shown to activate Akt pathway in ECs promoting their proliferation and motility, important initiating steps of angiogenesis***(Deregibus et al., 2003).***

In addition, using in vitro and in vivo models, CD40L was demonstrated to indirectly enhance angiogenesis, by inducing expression of proangiogenic factors including vascular endothelial growth factor (VEGF), and by upregulating, as mentioned above, expression and activity of various MMPs on ECs ***(Murugaiyan et al., 2007).***

The neo-vessels formed would enhance the inflammatory load of the lesion by acting as route of entry for immune cells. It is worth noting at this point, that this angiogenesis-inducing function of CD40L/CD40 dyad was proved important in other diseases, including tumors, where the new vessels would facilitate access of immune cells into the tumor site, but also would enhance the dissemination and spread of cancerous cells ***(Murugaiyan et al. 2007).***

Taken together, the above described processes would promote a destability of the fibrous plaque inducing as such lesion rupture or erosion at the endothelial layer ***(Hassan et al., 2012).***

Plaque rupture and athero-thrombosis

Athero-thrombosis is triggered by rupture of an unstable lesion, exposing thrombogenic factors in the lesion to various components in the circulation. Platelets and coagulation processes are activated leading to the formation of a clot. Indeed, a platelet plug is initiated by adhesion of platelets to extracellular matrix proteins of the ruptured plaque, mainly collagen, a process strengthened by other circulating protein and forming links between platelets and collagen fibrils. Activation of the coagulation factors occurs simultaneously, responding in a complex cascade to form fibrin strands which strengthen the platelet plug ***(Libby, 2000).***

At this level, CD40Lis implicated in athero-thrombosis via two different functions. On one hand, CD40L was shown to induce production of tissue factor, initiating the coagulation cascade, and on the other hand, CD40Lwas reported to play a role in platelet activation leading to platelet aggregation and thrombus formation. It is worth mentioning at this point that the release of thrombotic components upon lesion rupture would increase CD40L expression on platelets as well as the ultimate release of soluble CD40L, allowing as such further CD40Lmediatedevents to be implemented in vascular cells, leukocytes and in platelets themselves ***(Libby, 2000).***

CD40L-induced tissue factor expression.

The CD40L/CD40 interaction was shown to correlate with tissue factor production in the atherosclerotic lesion. Indeed, CD40 was shown to

colocalize with tissue factor in atherosclerotic plaques ***(Schonbeck et al., 2000).***

Moreover, CD40L was shown to enhance tissue factor production by many vascular cells in culture. For instance, monocytes/macrophages exhibited an increased tissue factor protein level and activity upon their stimulation with CD40L in culture ***(Mach et al., 1997a).***

Interestingly, the CD40L-induced tissue factor expression on monocytes/macrophages was recently shown to correlate with hypercholesterolemia, a risk factor of atherosclerosis ***(Sanguigni et al., 2005).*** Similarly, CD40L-stimulated ECs exhibited an increased procoagulant activity via an upregulation of their tissue factor expression and a downregulation of the blood coagulation inhibitor, thrombomodulin ***(Lutgens et al., 2007).***

As with other vascular cells, CD40L was also shown to induce in SMCs, the expression of a functional tissue factor capable of activating the coagulation cascade ***(Schonbeck et al., 2000).***

Biological role of CD40L in platelets.

As to the platelet-activating role of CD40L **(Fig. 12),** it has been described to implicate two or more different receptors. The group of ***André***, in 2002, presented in vitro and in vivo evidence showing the induction of platelet activation by CD40L interacting with the αIIbβ3 integrin ***(Andre et al., 2002b).***

In addition, they presented in vitro evidence showing the capacity of D40L/αIIbβ3interaction to induce activation and spreading of platelets as well as their aggregation under high shear conditions ***(Andre et al., 2002b).***

On the other hand, ***Inwald et al. (2003)*** have demonstrated the capacity of CD40L to activate platelets via its interaction withCD40 expressed on

platelets surface. Indeed, CD40L-stimulatedplatelets exhibited CD62P expression, dense granule and α-granule release, as well as pseudopodia formation and morphological changes, all markers of activation.

In addition, CD40L upregulated the formation of platelet–leukocytes aggregates which play an important role in the recruitment of leukocytes to the site of vessel injury. These CD40L-mediated effects were abrogated by antibodies directed against the CD40 receptor, rather thanthe αIIbβ3 integrin***(Inwald et al., 2003).***

In fact, ***Inwald et al. (2003)*** demonstrated that the CD40L/CD40 interaction was capable of activating β3. Therefore, the authors argued that CD40L-induced platelet activation and aggregation reported to be mediated via the αIIbβ3 integrin, is rather occurring by a CD40L/CD40 interaction, which would ultimately activate αIIbβ3 to enough levels under high shear stress, and cause platelet aggregation**.**

The role of CD40in CD40L-mediated platelet activation has been further shown in other studies**.** Interestingly, it is reported that sCD40L by interacting with CD40,is capable of potentiating agonist-induced platelet activation and aggregation, by causing platelet shape change and actin polymerization. Such response was abrogated when the CD40L/CD40binding was inhibited, by mutating CD40L residues implicated in the binding to CD40, or by deleting the CD40 gene ***(Hassan et al., 2012)***

In addition, it was revealed that the signaling pathway downstream of CD40Lstimulation involve TNF receptor-associated factor-2 (TRAF-2),Rac1, and p38 mitogen-activated protein kinase (MAPK) ***(Yacoubet al. 2010).***

These findings identify CD40L/CD40 interactions as enhancer rather than inducer of platelet function, but nevertheless provide further evidence as

to the important pro-thrombotic role ofCD40L acting particularly via its CD40 receptor ***(Hassan et al., 2012).***

On the other hand, still another receptor for CD40L, the α5β1 integrin is expressed on platelets surface and could be implicated in the role of CD40L in platelet activation. This possibility is currently under investigation. Therefore, CD40L is a platelet agonist that may act, depending on the settings, through a specific receptor without the others, or may implicate an interplay between different receptors, activating parallel pathways and leading to the cellular response***(Hassan et al., 2012)***.

CD40L and CAD Prognosis

A growing body of evidence has shed light on many aspects of the role of CD40L in cardiovascular disease, and it has been supported that it may have a predictive value ***(Dominguez-Rodriguez et al., 2009)***.

In the Women's Health Study, high sCD40Lplasma levels were associated with a higher risk of major cardiovascular events ***(Schonbeck et al., 2001).*** In the CAPTURE (c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina) trial, patients with high sCD40L levels had an almost3-fold higher risk for cardiovascular death or AMI, whereas in the MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study, highsCD40L was an independent risk factor for recurrent cardiovascular events ***(Heeschen et al., 2003) and (Kinlay et al., 2004).***

Indeed, it has been suggested that combined assessment of CD40L and troponin-T levels has better predictive value regarding AMI risk. Moreover, carriers of the -3459A>G polymorphism in theCD40L gene are at higher AMI risk, indicative of a causal role of sCD40L in the pathophysiology of ACS ***(Malarstig et al., 2006).*** Notably, a small prospective study suggested that highsCD40L levels predict angiographic restenosis in patients who undergo coronary angioplasty ***(L'Allier et al., 2005).***

CD40/CD40L as aTherapeutic Target in Atherosclerosis

Antiplatelet/antithrombotic agents:

As activated platelets are the main source of circulating sCD40L, antiplatelet agents were among the first drugs studied for their CD40Lloweringproperties. Clopidogrel, which acts via adenosine diphosphate receptor inhibition, completely inhibits sCD40L release from adenosine diphosphate-stimulated platelets; patients under clopidogrel treatment exhibit reducedCD40L platelet expression and circulating sCD40Llevels ***(Yip et al., 2006).***

Moreover, it was also found that both short- and long-term treatment with clopidogrel significantly reducessCD40L levels in patients with stable CAD ***(Azar et al., 2006)***, although another study demonstrated enhanced expression ofCD40L on platelets surface after 1 year of treatment with clopidogrel 75 mg/day plus aspirin 325 mg/day in patients with CAD ***(Saw et al., 2008).***

Cyclooxygenase-2 inhibition may also be a potential target. Patients receiving aspirin exhibit decreased sCD40Lrelease from platelets after platelet activation with collagen ***(Nannizzi-Alaimo et al., 2003).***

The GP IIb/IIIa receptor inhibitors, such as eptifibatide, abciximab, and tirofiban, inhibit platelet aggregation as well as sCD40L release in vitro

(Nannizzi-Alaimo et al., 2003).The beneficial effect of GP IIb/IIIa inhibitors has been also demonstrated in clinical studies. The GP IIb/IIIa antagonists appear to be particularly beneficial for the subgroup of high-risk ACS patients with increased sCD40L levels; abciximab significantly reduced cardiovascular risk in patients with elevatedsCD40L levels in the CAPTURE trial ***(Heeschen et al., 2003)***.

In addition, as increased sCD40L levels reflect a prothrombotic state, the FRISC II (Fragmin and fast Revascularization during InStability in Coronary artery disease II) study demonstrated hat low-molecular-weight heparin reduces the risk of AMI in non–ST-segment elevation ACS patients with high sCD40L levels ***(Pignatelli et al., 2004)and (Malarstig et al., 2006).***

Statins.

Multiple pleiotropic effects have been attributed to statins; in vitro studies have demonstrated that statins can also reduce oxidized low-density lipoprotein–induced or cytokine-induced CD40L expression on human umbilical vein ECs, SMCs, and mononuclear phagocytes in a concentration-dependent way ***(Schonbeck et al., 2002).***

Additionally, incubation of activated platelets with atorvastatin leads to decreased platelet-induced cycloxygenase-2 expression in human umbilicalvein ECs ***(Mosheimer et al. 2005).*** Therefore, statins may be actively implicated in platelet-endothelium interaction. Indeed, at a clinical level, statin treatment reduces plasma sCD40Llevels in patients with CAD ***(Li et al., 2004).***

Notably, the MIRACL study demonstrated that early atorvastatin treatment initiation after ACS reduces the risk for future cardiovascular events associated with high sCD40L levels; nevertheless, atorvastatin treatment only slightly affected sCD40Llevels***(Kinlay et al., 2004)***.

Thiazolidinediones.

Thiazolidinediones are peroxisome proliferator-activated receptor-γ agonists, widely used 2 diabetes mellitus treatment. Evidence from in vitro studies suggests that different cell types, such as platelets or ECs, treated with thiazolidinediones exhibit reduced expression of CD40L messenger ribonucleic acid or protein levels ***(Jiang et al., 2006).***

Rosiglitazone treatment for 12 weeks significantly reduced serum sCD40L levels in patients with type 2diabetes mellitus and CAD compared with placebo ***(Marx et al., 2003)***,although short-term rosiglitazone treatment failed to affectsCD40L levels in healthy persons, highlighting thecomplex mechanisms regulating sCD40L release in different disease states***(Hetzel et al., 2005)***.

Table 4. Approaches for targeting CD40–CD40L-mediated effects *(Rizvi etal., 2008).*

Tools	Mechanism of action	Effect
Small interfering RNA	Post-transcriptional gene silencing	Reduction of CD40 expression
Anti-CD40L antibody	Possible interruption of CD40–CD40L interaction	Inhibition of the inflammatory cascade by competition with CD40L
Estradiol	Binding to estrogen receptor a(ERa)	Reduction of IFN-g-induced CD40 and CD40L expression in endothelial cells

Antioxidantsand glycoprotein IIb/IIIa inhibitors	Redox scavengers and plateletinhibitors	Attenuation of platelet soluble CD40L release; counteraction of recombinant CD40L-mediated endothelial oxidative stress

Smoking and Cardiovascular Disease

Smoking is one of six major modifiable risk factors for CVD. Conversely, CVD is the leading cause of death from smoking. In some regions, including South America, Eastern Europe, and South-East Asia, there were approximately twice as many smoking-attributable deaths from CVD as there were from lung cancer or respiratory diseases during the same period. The healthcare costs associated with CVD are correspondingly large. Worldwide, the number of smokers continues to increase and is estimated to reach 1.7 billion by 2025 ***(Erhardt, 2009).***

Most of the risk of acute myocardial infarction (MI) associated with CVD can be explained by nine factors (the INTERHEART study),of which six increase the odds ratios (ORs) for MI and three decrease the ORs for MI. Of these factors, smoking is second only to dyslipidemia as a risk factor for MI ***(Yusuf et al., 2004).*** According to the Systemic Coronary Risk Evaluation (SCORE) project, the 10-year fatal cardiovascular risk is approximately doubled for smokers vs. nonsmokers for any given age, systolic blood pressure, and cholesterol level ***(Conroy et al., 2003).***

Negative effect of smoking	Cause/marker
Vascular dysfunction	↑ Vascular resistance ↓ Vascular compliance ↓ NO
Increased inflammation	↑ Homocysteine ↑ C-reactive protein ↑ Fibrinogen ↑ Interleukin-6
Progression of atherosclerosis	↑ LDL-C and total cholesterol ↓ HDL-C
Increased plaque development	↑ Triglycerides ↑ Lipid peroxidation
Development of thrombi	↑ Blood levels of catecholamines
Increased platelet activation and coagulation	↑ Blood levels of fibrinogen
Increased plaque instability	↑ Blood levels of metalloproteinases
Other potential mechanisms	↑ Oxidized LDL-C
Oxidative stress	↓ NO
Mitochondrial damage to heart muscle	↑ Mitochondrial DNA damage

Table 5: Negative vascular effects ofsmoking.
NO:Nitrous oxide; LDL-C:Low-density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol. ***(Erhardt, 2009).***

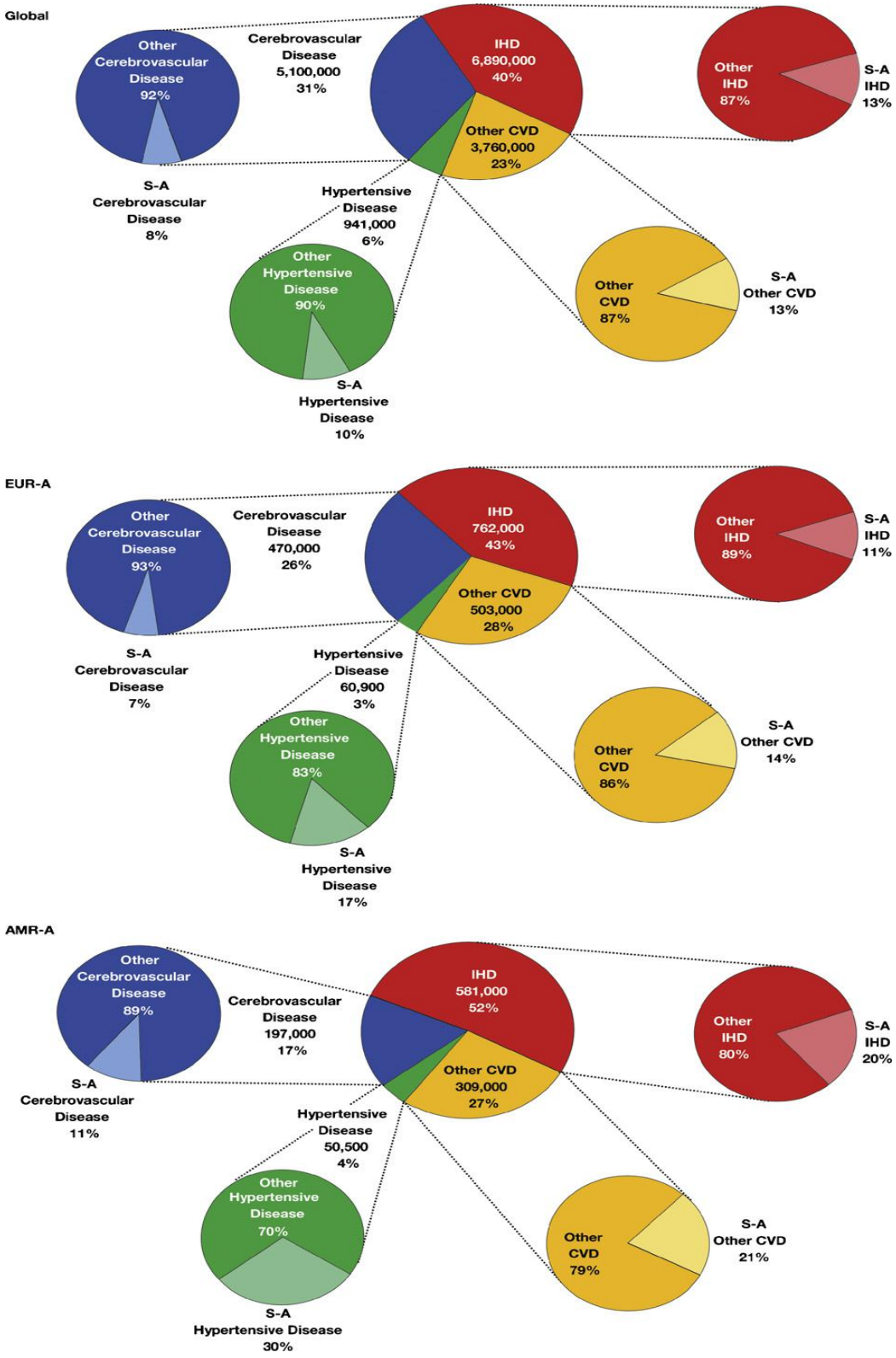

Fig. 14. Proportion of cardiovascular disease deaths attributable to smoking globally and in World Health Organization regions EUR-A (Western Europe) and AMR-A (North America) l Communication). CVD, cardiovascular disease; IHD, ischemic heart disease; S-A, smoking-attributable. ***(Erhardt, 2009).***

Smoking and platelet activation

Smoking activates platelets, increasing the risk of thrombus formation and leading to damage to the lining of the arteries, facilitating the development of atherosclerosis. Levels of platelet activation are higher in smokers than non-smokers ***(Brunner et al., 2005).***

In addition, fibrinogen, a mediator of platelet activation associated with an increased risk of heart disease, is increased in smokers. Thromboxane, a marker of platelet activation, has also been shown to increase in smokers but rapidly declines upon cessation, indicating that the increase is likely to be a direct result of smoking***(Hunter et al., 2001).***

Role of Thrombopeitin (TPO):

Thrombopoietin (TPO) is a humoral growth factor originally identified for its ability to stimulate the proliferation and differentiation of megakaryocytes**,** which is constitutively produced by the liver and kidneys, and is then cleared from circulation upon binding with its receptor, c-Mpl, expressed mainly on platelets and megakaryocytes ***(Kuter & Begley, 2002) (Kaushansky, 2003).***

Lupia et al. (2010) suggested the involvement of Thrombopoietin (TPO) in the pathophysiology of enhanced platelet activation in cigarette smoking. They found that chronic smokers have higher circulating TPO levels than nonsmokers, and higher platelet–leukocyte binding and platelet P-selectin expression ex vivo. TPO present in the plasma of cigarette smokers also primed platelet activation and platelet–monocyte interaction, which may have a pro-atherosclerotic and pro-thrombotic role in this condition.

Several studies have shown that TPO also directly modulates the homeostatic potential of mature platelets by influencing their response to several stimuli. In particular TPO, which does not induce platelet aggregation per se, enhances platelet activation in response to different

agonists and the subsequent platelet–leukocyte adhesion via P-selectin ***(Tibbles et al., 2002).***

Recent studies demonstrated that higher circulating TPO levels are associated with increased markers of platelet activation in vitro in patients with unstable angina and burned patients who develop sepsis, suggesting that TPO may enhance platelet function in the early phases of these diseases ***(Lupia et al., 2010).***

Interference with platelet function.

An alternative mechanism to explain the link between cigarette smoking and cardiovascular disease may be by interfering with platelet function. Smokers have reduced platelet survival, greater platelet turnover and aggregation, and abnormalities in thromboxane and prostacyclin metabolism. Increased levels of the platelet alpha granule matrix component beta thromboglobulin, and increased levels of a granule membrane component soluble P-selectin in smokers' plasma also implies inappropriate platelet activation ***(Erhardt, 2009).***

SUBJECTS AND METHODS

1. Subjects

The present study was conducted on 79 subjects,59 males and 20 females from April 2012 to May 2013, subdivided into two groups.

Group I: This group included 60 patients with AMI (ST segment elevation and non-ST segment elevation) with their mean age (± SD) about 51.32 years (± 6.03). They were 45 males and 15 females that had been admitted to Coronary Care Unit in Benha University Hospital.

Table (6): Distribution of the patient group according to the admission diagnosis.

Admission diagnosis among patient group	No	%
ST segment elevation MI (STEMI)	33	55.0
Non - ST segment elevation MI (NSTEMI)	27	45.0
Total	60	100.0

Exclusion criteria:

1- Those patients with acute MI onset > 24 h, or age > 60 years.

2- Patients with history of Percutaneous Coronary Intervention (PCI), or coronary artery bypass graft surgery.

3- Patients with diabetes mellitus, dyslipidaemia, atrial fibrillation, renal or hepatic failure, significant valvular abnormalities, chronic obstructive pulmonary disease, active inflammatory or connective tissue diseases, malignancy , febrile disorders, cardiogenic shock, patients requiring intra-aortic balloon pump therapy and patients with a history of recent surgery or trauma within the preceding 2 months .

4- Patients receiving anti-platelet agents such as acetylsalicylic acid and clopidogrel for any medical reason. Also, the patients that were given these agents or heparin during transfer to the hospital were excluded.

Inclusion criteria:

1- Patients of both genders with age range (40 – 60) years with acute MI defined as having symptoms of ischemia that were verified by electrocardiography or by increased levels of biochemical markers (Creatine Kinase-MB isoenzyme [CK-MB], >25 U/L [6.6 ng/mL] or troponin I [TnI], >0.1 μg/L [0.1 ng/mL])***(Apple et al., 1995).***

2- Patients who were undergoing primary Percutaneus Coronary Intervention were included in the study.

Group II: This group included nineteen apparently healthy individuals of matched age and sex. Their mean age (±SD) was 50.47 years (± 4.33). They were 14 males &5 females.

Table (7):Distribution of the studied groups according to gender.

Gender		group		Total
		control group	Patient group	
Male	Number	14	45	59
	% within group	68.4%	75.0%	73.4%
Female	Number	5	15	20
	% within group	31.6%	25.0%	26.6%
Total	Number	19	60	79
	% within group	100.0%	100.0%	100.0 %

Both groups were further subdivided into 2 subgroups:

❖ A-*Smokers group* defined as those currently smoking any type of tobacco in more than ten cigarettes per day and ex-smoker patients were excluded from the study.

❖ B- *Non-smokers group*.

Table (8): Comparison between the studied groups as regards smoking status.

Group / Smoking Status	Patient	Control	Total
Smokers			
No.	30	10	40
%	50	52.6	50.6
Non-smokers			
No.	30	9	39
%	50	47.4	49.4

2. Methods

All individuals in the study were subjected to the following:

1- Full history taking paying attention to:

* Name, age and date of admission.

* Risk factors:

Smoking, number of cigarettes per day

*History of chest pain.

2- Chest pain was confirmed with E.C.G. or diagnostic coronary angiography findings.

ST segment elevation MI

- Initial ST-segment elevation or a LBBB pattern is strongly correlated with an acute occlusive obstruction of an epicardial vessel and suggests potential benefit from reperfusion therapy ***(Menown et al., 2000)*.**
- By emergency coronary angiography. demonstrating an acutely occluded infarct vessel, with the characteristic appearance of thrombus or a cutoff sign at the point of occlusion, coupled with left ventriculography to ascertain the segmental wall motion profile***(Simes et al., 1995)*.**

Non ST segment elevated myocardial infarction

In NSTEMI, ST depression (or transient ST elevation) and T wave changes occur in up to 50% of patients ***(Kleiman et al., 2002)*.**

3-Laboratory investigations:

Specimen collection:

Blood samples were collected from every participant in the study. Initial blood samples were collected from patients once admitted to the emergency department and only those with confirmed diagnosis of MI were included in the study. At first seven milliliters of venous blood were collected under complete aseptic precautions, divided into two tubes:

a) 1.8 ml into citrate test tubes were centrifuged immediately at 1500 rpm for 10 min & assayed for Prothrombin Time.

b) 2 ml into EDTA test tubes were centrifuged immediately at 1500 rpm for 10 minutes. The separated plasma was stored at - 20° C for subsequent assay of sCD40L.

c) The rest into plain test tubes without anticoagulant were left till coagulation. After coagulation, samples were centrifuged (at 1500 rpm for 15 minutes). The separated serum was divided into two aliquots.

1. One was designated for the immediate assay of liver function tests, kidney function test and cardiac enzymes (CK-MB and Troponin I).

2. The second aliquot was stored at -20ºC for subsequent assay of HS-CRP.

The second venous blood samples (2 ml) were collected from the patients admitted to the coronary care unit in Banha University Hospital after fasting for 8-10 hours under complete aseptic precautions in Na Fluoride serum test tubes, centrifuged at 1500 rpm for 10 minutes. The separated serum is used for the assay of fasting blood sugar. The third venous samples (2 ml) were collected after fasting for 12-14 hours under complete aseptic precautions in plain test tubes without anticoagulant. The plain test tubes were left till coagulation. After coagulation, samples were centrifuged (at 1500 rpm for 15 minutes). The separated serum was used for the assay of lipid profile. Hemolysed samples were discarded. Repeated freezing and thawing was avoided.

Two milimeters of blood were collected from apparently healthy individuals after fasting for 8-10 hours under complete aseptic precautions into in Na Fluoride serum test tubes, then handled in the same way as the patients' samples and used for the assay of fasting blood sugar. Eight milliliters of venous blood then were collected after fasting for 12-14 hours under complete aseptic precautions, then handled, divided in the same way as the patients' samples.

The laboratory investigations *included*:

A) Routine Laboratory Investigations:

*** Fasting blood glucose level**

*** Liver Function Tests** (Alanine Transaminase, Aspartate Transaminase, Total Bilirubin, Albumin and Prothrombin Time).

*** Kidney Function Tests** (Urea and Creatinine).

*** Lipid Profile** (Total Cholesterol, High-denisty Lipoprotein Cholesterol, Low-denisty Lipoprotein Cholesterol and Triglycerides).

B) Specific Laboratory Investigations:

*** Cardiac Markers** (CK-MB & Troponin I)

*** High sensitive C-reactive protein (CRP)** using a specific enzyme-linked immunosorbent assay (ELISA) kit.

*** The concentration of soluble CD40L levels** in plasma using a specific enzyme-linked immunosorbent assay (ELISA) kit.

Analytical Methods:

1. Blood Glucose Level:

The analysis was done using Biosystem A15 auto-analyzer applying glucose oxidase enzymatic colorimetric method.

In this method, oxidation occurs in the presence of glucose oxidase ***(Carl et al., 2006)*.**

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucoseoxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4 - \text{aminophenazine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4H_2O$$

Red color was measured at 546 nm.

2. Total Cholesterol (TC)*:*

Total cholesterol was assayed on Biosystem A15 auto-analyzer applying an enzymatic colorimetric method. In this method, cholesterol esterase (CE) hydrolyzes cholesterol esters to free cholesterol and fatty acid ***(Dietschy et al., 1976).***

$$\text{Cholesterol ester} + H2O \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O2 \xrightarrow{\text{Cholesterol oxidase (COX)}} \text{cholesterol 4en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-aminoantitpyrine(4-AAP)} + \text{phenol} \longrightarrow \text{quinoneimine} + H_2O$$

Red color was measured at 546 nm .

3. Triglycerides (TG):

The analysis of TG was done using the Biosystem A15 auto-analyzer applying an enzymatic colorimetric method ***(McGowan et al., 1983).***

$$\text{Triglycerides} \xrightarrow{\text{lipase}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerokinase (GK), } Mg^{++}} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[\text{(GPO)}]{\text{Glycerophosphate oxidase}} \text{hydroxy-acetatephosphate + } H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP + Phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine + } H_2O$$

Red color was measured at 546 nm.

4. High Density Lipoproteins (HDL-C):

The HDL-C was assayed on the Biosystem A15 autoanalyzer after precipitation of LDL and VLDL by dextran sulfate and magnesium in the separating reagent. The LDL and VLDL portions are then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant was assayed with an enzymatic reagent. HDL cholesterol reagent was used to measure the cholesterol concentration by a timed endpoint method ***(Assman et al., 1983).***

5. Low Density Lipoproteins (LDL-C):

LDL-C value was calculated according to "Friedwald's equation":

LDL-C = Total cholesterol-(HDL-C+TG/5)

This equation is applied provided that serum TG is < 400 mg/dl ***(Friedwald et al., 1972).***

6-Creatinine:

The analysis was done using Biosystem A15 auto-analyzer applying modified jaffé reaction

$$\xrightarrow{\text{Alkaline solution}}$$

Creatinine+picric acid creatinine picric acid complex.

As alkaline solution creatinine combines with picric acid to form orange red colored complex. The absorbance increase proportional to conc. of creatinine **(*Myers et al., 2006)*.**

7- Urea

The analysis was done using Biosystem A15 auto-analyzer applying a coupled enzymatic method described below. Urea concentration is determined from the rate of decreased absorbance at 340 nm due to conversion of NADH to NAD that can be measured by spectrophotometry ***(Gutmann&Bergmeyer., 1974).***

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{\text{Glutamate dehydrogenase}} \text{Glutamate} + NAD^+$$

8- ALT

The analysis was done using Biosystem A15 auto-analyzer applying kinetic method.

The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxaloglutarate yielding pyruvate and L-glutamate.The catalytic concentration is determined from the rate of decreased absorbance at 340 nm due to conversion of NADH to NAD by lactate dehydrogenase coupledreaction***(Gella et al., 1985).***

$$\text{L-alanine} + \text{2-oxaloglutarate} \xrightarrow{\text{ALT P-5-P}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H \xrightarrow{\text{LDH}} \text{Lactate} + NAD$$

9-AST

The analysis was done using Biosystm A15 auto-analyzer applying kinetic method.

The amino group is enzymatically transferred by AST present in the sample from aspartate to the carbon atom of 2-oxaloglutarate yielding oxaloacetate and L-glutamate.The catalytic concentration is determined from the rate of decreased absorbance at 340 nm due to conversion of NADH to NAD by malate dehydrogenase coupled reaction ***(Gella et al., 1985).***

Aspartate + 2-oxaloglutarate $\xrightarrow{\text{AST P-5-P}}$ Oxaloacetate+ L-Glutamate

Oxaloacetate + NADH + H $\xrightarrow{\text{MDH}}$ Malate + NAD

10- Total Bilirubin

The analysis was done using A15 system auto-analyzer based on Jendrassik and Grof bilirubin method. Sulphanilic acid is diazotized by the nitrous acid pro-duced from the reaction between sodium nitrite and hydrochloric acid.Bilirubin reacts with the diazotized sulphanilic acid (diazo reagent) to form azobilirubin. Caffeine is an accelerator and gives a rapid and complete conversion to azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate reagent and the absorbance of the blue-green solution is read in a colorimeter using an orange filter 590 nm or in a spectrophotometer at wavelength 600 nm ***(Pearlman and Lee., 1974).***

11-Albumin

The analysis was done using A15 system auto-analyzer based on binding of albumin to the indicator dye bromocresol green (BCG) in pH 4.1 to form a blue-green colored complex. The intensity of the blue green color is directly proportional to the concentration of albumin in the sample. It is determined by monitoring the increase in absorbance at 623 nm, or 578 nm***(Doumas, Watson & Biggs, 1971).***

Albumin + BCG $\xrightarrow{\text{pH 4.1}}$ Albumin-BCG-Complex

12-PT

The PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of the tissue extract (thromboplastin) ***(Ingram and Hills., 1976).***

0.1 ml of plasma was deliverd into a glass tube placed in a waterbath, allowed to warm for 1-3 min and then 0.2 ml of warmed PT reagent (containing thromboplastin and calcium chloride) was added to the plasma, well mixed and timming was started immediately to record the endpoint of coagulation.

13- CK-MB

The analysis was done using Biosystem A15 auto-analyzer applying a modification of the IFCC method . The CK-MB reagent contains an antibody which binds to the M subunit of CK in the serum sample thereby inhibiting the activity of the M subunit. The remaining activity, corresponding to CK-B fraction activity is measured using according to IFCC reference method for measuring CK activity. CK-MB activity is then obtained by multiplying by 2 the remaining activity.

Creatinephosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP + glucose $\xrightarrow{HK \quad Mg^{++}}$ glucose-6-phosphate + ADP

Glucose-6-phosphate + NADP $\xrightarrow{G6PD}$ 6-phosphogluconate + NADPH

The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK-MB in the sample ***(Horder et al., 1991).***

14- Troponin I

Troponin I concentrations were measured on the mini VIDAS instrument using VIDAS Troponin I Ultra (TNIU) kit provided by bioMerieux, Inc. Box 15969, Durham, NC 27704-0969/ USA for determination of human cardiac troponin I in human serum or plasma using the ELFA technique.

A) Assay principle ***(WU et al., 1998)***

The test combines a one step immunoassay sandwitch method with a final fluorescent detection (ELFA).The solid phase Receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay.

The sample is transferred into the wells containing anti-cardiac troponin antibodies labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture is cycled in and out of the SRP several times. This operation enables the troponin I to bind with the immunoglobulins fixed to the interior wall of the SRP and the conjugate to form a sandwich. Unbound components are eliminated during washing steps.

Two detection steps are then performed successively. During each step, the substrate (4-Methyl-umbelliferylphosphate) is cycled in and out of the SRP. The conjugate enzyme catalyzes the hydrolysis of this substrate into a product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450nm. The intensity of the fluorescence is proportional to the concentration of the antigen present in the sample**.**

B) Procedure:

1. The required reagents were removed from the refrigerator.

2. TNIU was typed or selected on the instrument to enter the test code. The calibrators was identified by S1 and S2, and tested in duplicate. The controls were identified by C1 and C2 and tested.

3. The calibrators, controls and samples were mixed using a Vortex-type mixer.

4. 200 µl of calibrator, control or samples were pipetted into the sample wells.

5. The SPRs and strips were inserted into the instrument.

7. The assay was iniated immediately. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 20 minutes.

8. After the assay was completed, the SPRs and strips were removed from the instruments.

9. Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the reagent strips's reading cuvette for each sample tested.

10. The results are automatically calculated by the instrument and the concentrations are expressed in ng/ml.

15- Measurment of sCD40 ligand

sCD40 ligand was measured using eBioscience human ELISA kits provided by Bender MedSystems GmbH Campus Vienna Biocenter 2 1030 Vienaa, Austria. This kit was designed for determination of sCD40 ligand in human plasma using sandwich technique.

1) PRINCIPLE OF THE TEST: ***(Burtis et al., 2008)***

An anti-human sCD40L coating antibody is adsorbed onto microwells. Human sCD40L present in the sample or standard binds to antibodies adsorbed to the microwells and the HRP-conjugated antihumansCD40L antibody is added and binds to human sCD40L captured by the first antibody.

Following incubation unbound HRP conjugated anti-human sCD40L is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of human sCD40L present in the sample or standard.

The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 humansCD40L standard dilutions and humansCD40L concentration determined using the standard curve.

2) REAGENTS SUPPLIED

- 1 aluminium pouch with a Microwell Plate coated with monoclonalantibody to human sCD40L.
- 1 vial (200 µl) HRP-Conjugate anti-human sCD40L monoclonalantibody.
- 2 vials human sCD40L Standard lyophilized, 20 ng/ml uponreconstitution.
- 1 vial Control high, lyophilized
- 1 vial Control low, lyophilized
- 1 vial (12 ml) Sample Diluent
- 1 vial (5 ml) Assay Buffer Concentrate 20x
  (PBS with 1% Tween 20 and 10% BSA)
- 1 bottle (50 ml) Wash Buffer Concentrate 20x
  (PBS with 1% Tween 20)
- 1 vial (15 ml) Substrate Solution (tetramethyl-benzidine)
- 1 vial (15 ml) Stop Solution (1M Phosphoric acid)
- 1 vial (0.4 ml) Blue-Dye
- 1 vial (0.4 ml) Green-Dye
- 2 Adhesive Films
- 2 Dilution Plates
- Product data sheet + certificate of analysis: 1 picture of product data sheet and certificate of analysis were provided.

3) PREPARATION OF REAGENTS

- All reagents were brought to room temperature prior to use. Only the appropriate quantity of reagents for the test were prepared

- **Wash Buffer (1x)**

  50 ml of the wash buffer concentrate were added to 950 ml of D.W.

- **Assay Buffer (1x)**

  5 ml of the assay buffer concentrate were added to 95 ml of distilled water.

- **HRP-Conjugate**

120 µl of the HPR-Conjugate were added to 11.88 ml of the assay buffer.

- **Human sCD40L Standard**

Lyophilized human sCD40L standard was reconstituted with distilled water.

- **Controls**

150 µl distilled water were added to lyophilized controls.

4) PREPARATION OF SAMPLES :

- Samples were left to thaw just prior to the assay
- They were mixed thoroughly
- Hemolyezed and lipemic samples were excluded

5) PREPARATION OF STANDARDS :

- 7 tubes were labelled, one for each standard point.

  S1, S2, S3, S4, S5, S6, S7
- Then 1:2 serial dilutions were prepared for the standard curve as follows:
- 225 µl of Sample Diluent were pipette into each tube.
- 225 µl of reconstituted standard (concentration of standard =20 ng/ml) were pipetted into the first tube, labelled S1, and then mixed (concentration of standard 1 = 10 ng/ml).
- 225 µl of this dilution were pipetted into the second tube, labelled S2, and mixed thoroughly before the next transfer.
- Serial dilutions were repeated 5 more times thus creating the points of the standard curve. Sample Diluent serves as blank.

Table (9): Serial sCD40L standards dilutions.

Standard	Human sCD40L Concentration (ng/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	10.00	1.997 1.960	1.979	1.3
2	5.00	1.194 1.210	1.202	0.9
3	2.50	0.713 0.755	0.734	4.0
4	1.25	0.385 0.369	0.377	3.0
5	0.63	0.225 0.222	0.224	0.9
6	0.31	0.122 0.122	0.122	0.0
7	0.16	0.076 0.074	0.075	0.5
Blank	0	0.029 0.026	0.028	5.4

6) ASSAY PROCEDURE:

1. Dilution plate was removed from pouch.

2. The number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards was determined.

3. 100 µl of the sample Diluent were added to the blank well of the dilution plate.

4. 100 µl of the standard dilutions (S1 - S7) were pipitted, in duplicate, in the standard wells

4. 80 µl of the sample Diluent were added to sample wells of the dilution plate.

5.20 µl of samples were added to designated sample wells of the dilution plate.

6. HRP-Conjugate was prepared.

7. 100 µl of HRP-Conjugate were added to all wells of the dilution plate.

8. Coated microwell strips were removed from aluminium pouch.

9. Coated microwell strips were washed twice with Wash Buffer.

10. 150 µl of reaction mix were transferred from dilution plate to coated microwell strips.

11. Microwell strips were covered and incubated 2 hours at room temperature (18° to 25°C) on a rotator set at 100 rpm.

12. Microwell strips were emptied and washed3 times with Wash Buffer.

13. 100 µl of TMB Substrate Solution were added to all wells.

14. The microwell strips were incubated for about 10 minutes at room temperature (18°to 25°C).

15. 100 µl Stop Solution were added to all wells.

16. Microwell reader was blanked and colour intensity was measured at 450 nm.

7) CALCULATION OF RESULTS :

The standard curve was constructed by plotting the mean absorbance (Y) of standards against log of the known concentration (X) of standards, using the four-parameter algorithm. Results were reported as concentration of sCD40L (ng/ml) in samples.

The measured concentrations of samples calculated from the standard curve were multiplied by their respective dilution factor (x 5), because samples have been diluted prior to the assay.

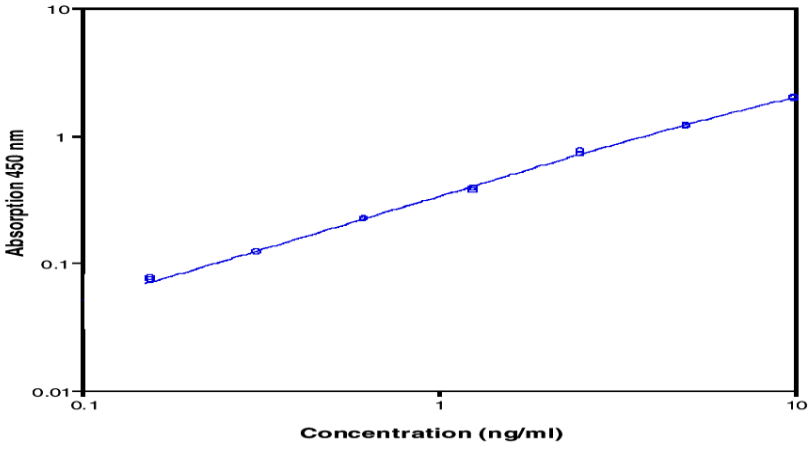

Figure (15) Human sCD40L ELISA Standerd Curve

16-Measuremet of high sensitivity C - reactive protein (HS-CRP) ELISA:

Highly sensitive CRP was measured using STAT-FAX reader using Accu-bind ELISA kits provided by Monbind Inc. lake fores, CA92630 USA. The kit was designed for determination of highly sensitive CRP in human in serum or plasma by microplate immunenzymatic assay.

1) PRINCIPLE OF THE TEST : (*Robert et al., 2000)*

The HS-CRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solidphase immobilization (on the microtiter wells).

A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45-minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies.

A tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of CRP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm**.**

2) REAGENTS SUPPLIED :

1. Antibody-coated wells (1 plate, 96 wells)

Microtiter wells coated with mouse monoclonal anti-CRP.

2. Reference standard set (1.0 ml/vial)

Contains 0, 0.005, 0.010, 0.025, 0.050 & 0.100 mg/l CRP in serum based buffer-BSA solution with prservatives.

3. HS-CRP Samlpe Diluent (50 ml/vial)
contains phosphate buffer-BSA solution with preservatives.

4. CRP Enzyme Conjugate Reagent (12 ml/vial)
Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives

5. TMB Reagent (11 ml/ bottle)
Contains one-step TMB solution.

6. Stop solution (1 bottle, 11 ml/bottle)
Contains diluted hydrochloric acid (1N HCl).

3) REAGENT PREPARATION :

All reagents were allowed to reach room temperature (18-25°C) before use.

Standards were not diluted.

Sample Preparation:

- Samples were left to thaw just prior to the assay
- They were mixed thoroughly
- Hemolyezed, lipemic or turbid samples were excluded.
- Patient serum was diluted 100 fold prior to use by mixing 5µl of serum with 495 µl sample diluents.
- Samples with expected CRP concentrations over 10 mg/l may were diluted (10 fold) of the 100-fold diluted solution with sample diluents (i.e. 10 µl of the 100-fold diluted sample to 90 µl sample diluents).

4) ASSAY PROCEDURE :

1. The desired number of coated wells in the holder were secured.

2. 10 µl of CRP standards, diluted specimens, and diluted controls were dispensed into the appropriate wells.

3. 100 µl of CRP Enzyme Conjugate Reagent were dispensed into each well.

4. All mixed thoroughly for 30 seconds.

5. All incubated at room temperature (18-25 °C) for 45 minutes.

6. The incubation mixture is removed by flicking plate contents into a waste container. The microtiter wells were rinsed and flicked 5 times with deionized or distilled water.

7. The wells were striked sharply onto absorbent paper or paper towels to remove all residual water droplets.

8. 100 µl TMB solution were dispensed into each well and gently mixed for 5 seconds.

9. All incubated at room temperature for 20 minutes.

10. The reaction is stopped by adding 100 µl of stop solution to each well.

11. Mixing gently for 30 seconds. All the blue color should have been changed to yellow color completely.

12. Absorbance at 450 nm was read with a microtiter well reader within 15 minutes.

5) CALCULATION OF RESULTS :

1. The mean absorbance value (OD 450) for each set of reference standards, controls and samples was calculated.

2. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in mg/l on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, the corresponding concentration of CRP (mg/l) was determined from the standard curve.

4. The obtained values of the patient samples and control sera were multiplied by the dilutionfactor of 100 to obtain CRP results in mg/l.

Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 16 soft ware. Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (X^2), student "t" test and Spearman's correlation coefficient (r) were used as tests of significance, stepwise multiple regression analysis was used to detect the significant predictors of PEDF level and design a linear equation to predict values of PEDF for given values of the predictors. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

P value >0.05 insignificant

P<0.05 significant

P<0.01 highly significant

***(Edward and Schultz., 1999*)**

RESULTS

The results of the present study were statistically analyzed, summarized and presented in 8 tables and 16 figures.

Table (10): Comparison between studied groups as regards the routine laboratory investigations.

Group / Variable	Patient Group Mean ± SD	Control group Mean ± SD	P value
FBS mg/dl	88.67±13.87	85.58±10.56	0.376 NS
Choesterol mg/dl	181.3±12.99	179.89±5.8	0.65 NS
Triglycerides mg/dl	141.95±6.79	139.32±3.38	0.108 NS
HDL-C mg/dl	51.7±4.67	51.37±3.25	0.98 NS
LDL-C mg/dl	143.67±6.66	140.37±2.11	0.062 NS
AST U/L	26.58±7.54	24.74±8.32	0.367 NS
ALT U/L	25.45±6.82	23.58 ± 6.54	0.296 NS
Albumin g/dl	4.1 ± 0.39	4.2 ± 0.31	0.235 NS
Bilirubin mg/dl	0.77 ± 0.19	0.72 ± 0.17	0.268 NS
PT INR	1.07425 ± 0.08	1.045 ± 0.35	0.093 NS
Creatinine mg/dl	0.78 ± 0.23	0.76 ± 0.26	0.746 NS
Urea mg/dl	27.6 ± 7.8	27.37 ± 5.56	0.905 NS

❖ There is no significant difference between both groups as regards the mean fasting blood sugar, total Cholesterol, Triglycerides, HDL-cholesterol, LDL-cholesterol, AST, ALT, Albumin, Bilirubin, INR, Creatinine, and Urea levels.

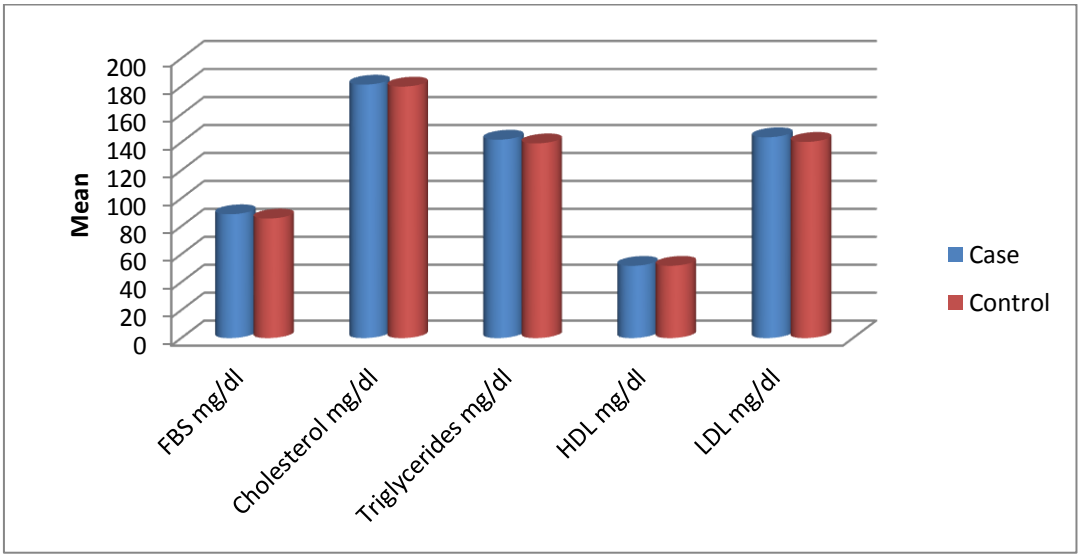

Figure (16): Comparison between studied groups as regards fasting blood sugar and Lipid profile.

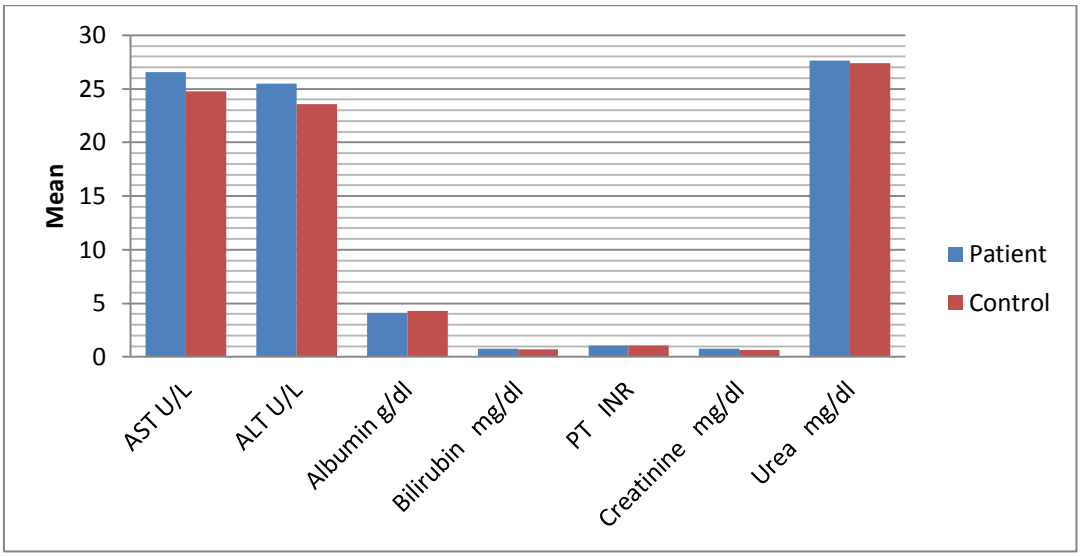

Figure (17): Comparison between studied groups as regards Kidney Function Tests & Liver Function Tests.

Reference Values:

Fasting blood sugar (FBS): 74-100 mg/dl, Total Cholesterol : less than 200 mg/dl, Triglycerides : less than 150 mg/dl, Low-density lipoprotein Cholesterol (LDL-Ch): less than 150 mg/dl, High-density lipoprotein Cholesterol (HDL-Ch): more than 50 mg/dl, AST: male <35, female <31 U/l; ALT: male <45, female <34 U/L; Albumin =3.5-5.2 g/dl, Total Bilirubin = 0-2 mg/dl, PT =12-14 seconds, Creatinine : male= 0.9-1.3, female =0.6-1.1 mg/dl. ***(Roberts et al., 2008).***

Table (11): Comparison between studied groups as regards myocardial markers, HS-CRP & sCD40L.

Variable	Patient	Control	P value
Troponin I ng/ml	1.16 ± 0.55	0.014± 0.005	0.001 HS
CK-MB U/L	227.15± 92.29	13.74± 5.67	0.001 HS
HS-CRP mg/L	5.98 ± 2.72	0.83 ± 0.33	0.001 HS
sCD40L ng/ml	9.45 ± 11.03	2.23 ± 1.57	0.006 HS

- There is a statistically highly significant increase in the mean Troponin I, CK-MB, HS-CRP and sCD40L concentration in patient group versus control group.

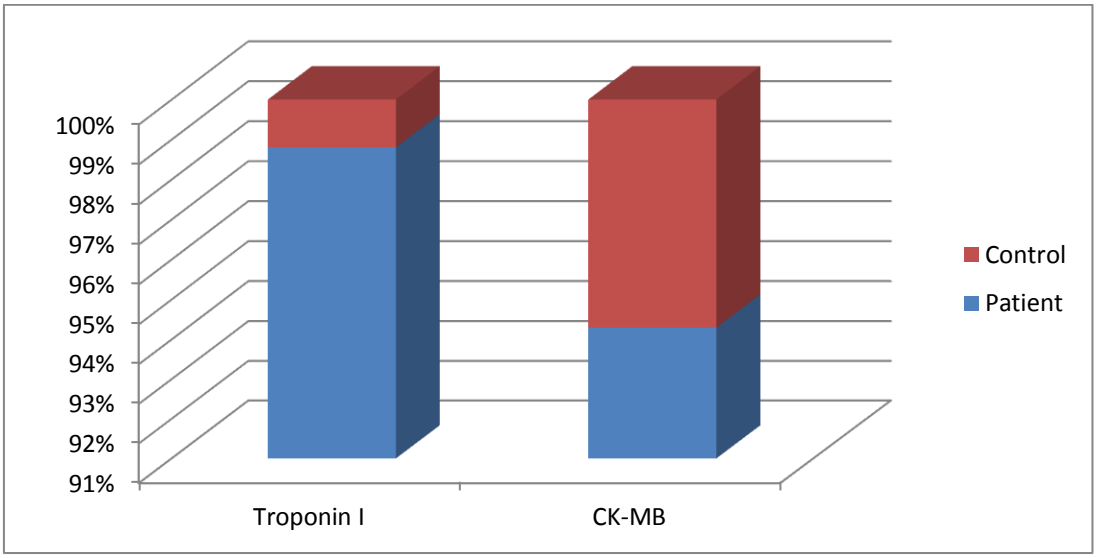

Figure (18): Comparison between studied groups as regards Cardiac markers.

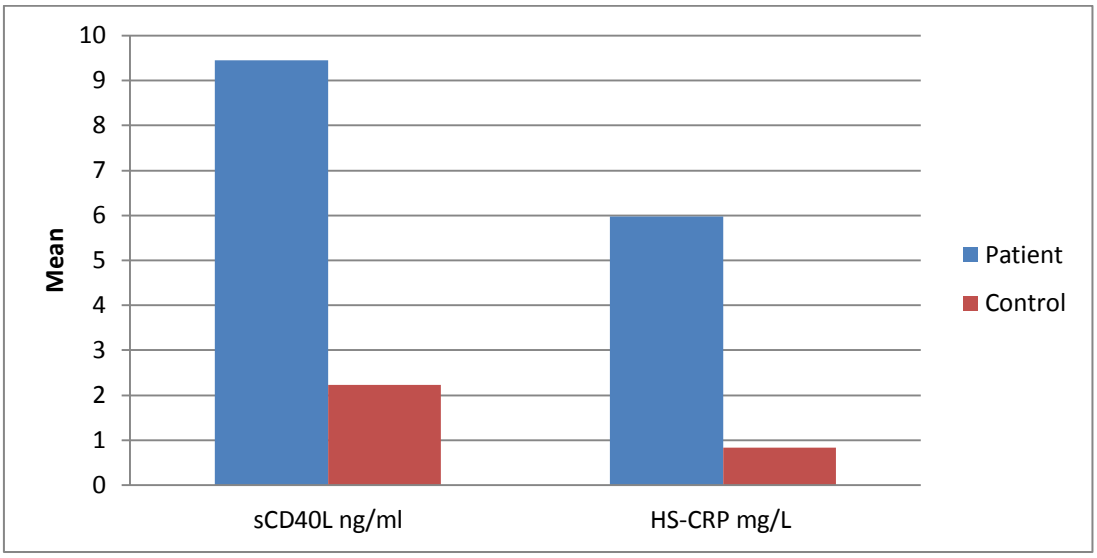

Fig (19): Comparison between the studied groups as regards HS-CRP & sCD40L.

Reference Intervals: Troponin I < 0.1 ng/ml, CK-MB < 25 U/L, HS-CRP: 0.2-6.3 mg/L, sCD40L: up to 3.78 ng/ml ***(Roberts et al., 2008).***

Table (12): Comparison between smokers and non-smokers as regards myocardial markers, HS-CRP and sCD40L.

Group / Variable	Smokers (*n.* 30) Mean ± SD	Non-smokers (*n.* 30) Mean ± SD	P value
Troponin I ng/ml	1.38 ± 0.45	0.93 ± 0.55	0.001 HS
CK/MB U/L	252.57 ± 73.28	201.73 ± 103.07	0.032 S
HS-CRP mg/L	6.97 ± 2.72	5.05 ± 2.41	0.005 HS
sCD40L ng/ml	12.49 ± 13.16	6.4 ± 3.44	0.018 S

Table (13): Comparison between patients with STEMI and those with NSTEMI as regards myocardial markers, HS-CRP and sCD40L.

Group / Variable	STEMI (*n.* 33) Mean ± SD	NSTEMI (*n.* 27) Mean ± SD	P value
Troponin I ng/ml	1.56 ± 0.45	0.66 ± 0.33	0.001 HS
CK/MB U/L	296.67 ± 31.91	142.19 ± 66.68	0.001 HS
HS-CRP mg/L	6.8 ± 2.67	4.99 ± 2.46	0.009 HS
sCD40L ng/ml	12.48 ± 13.73	5.72 ± 3.39	0.006 HS

❖ There is a statistically significant increase in the mean Troponin I, CK-MB, HS-CRP, sCD40L levels in smokers versus non-smokers and also in patients with STEMI versus those with NSTEMI.

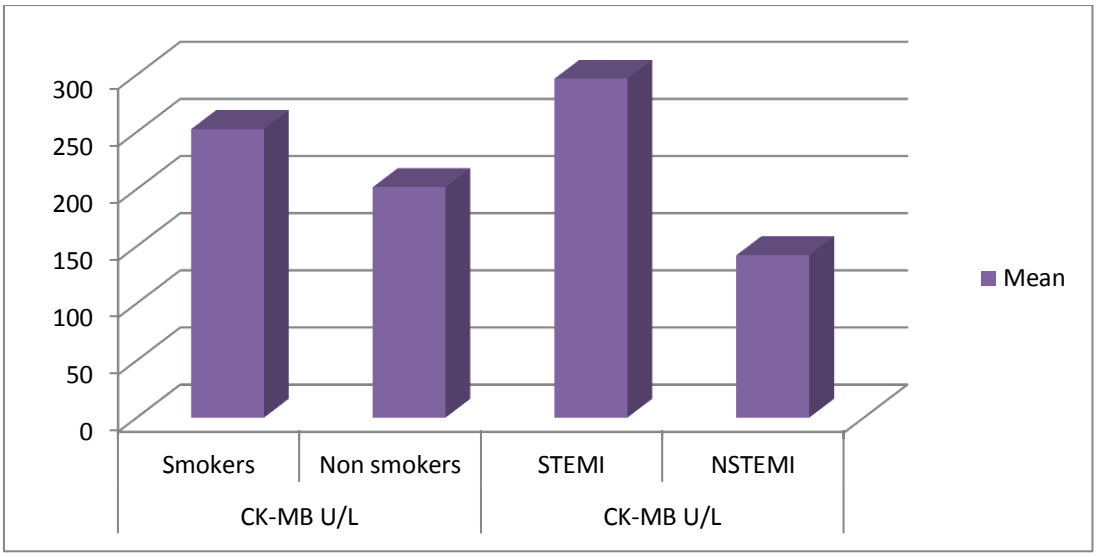

Fig (20): Comparison between the patient subgroups as regards CK-MB.

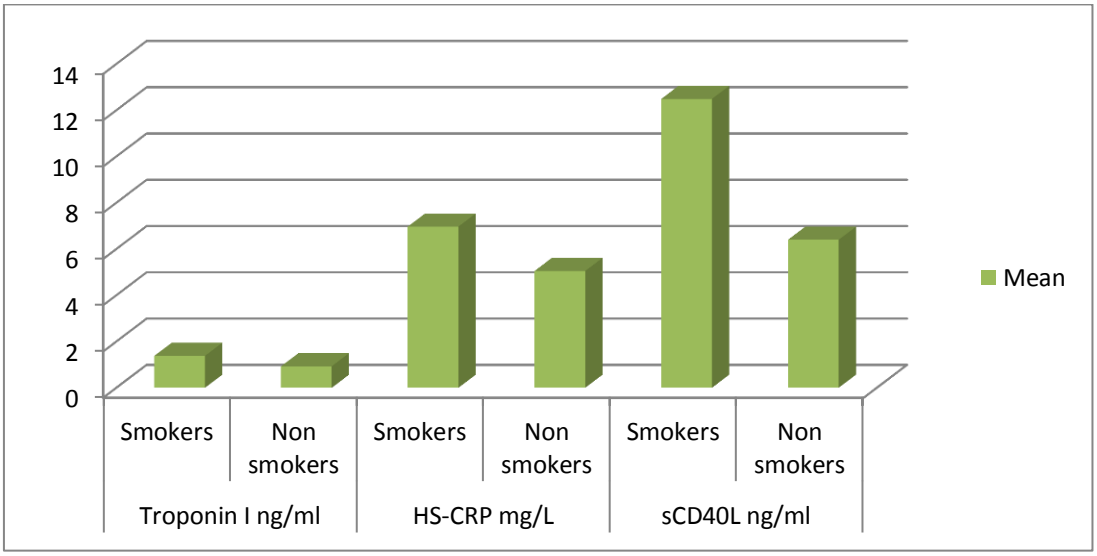

Fig (21): comparison between smokers and non-smokers as regards Troponin I, HS-CRP and sCD40L.

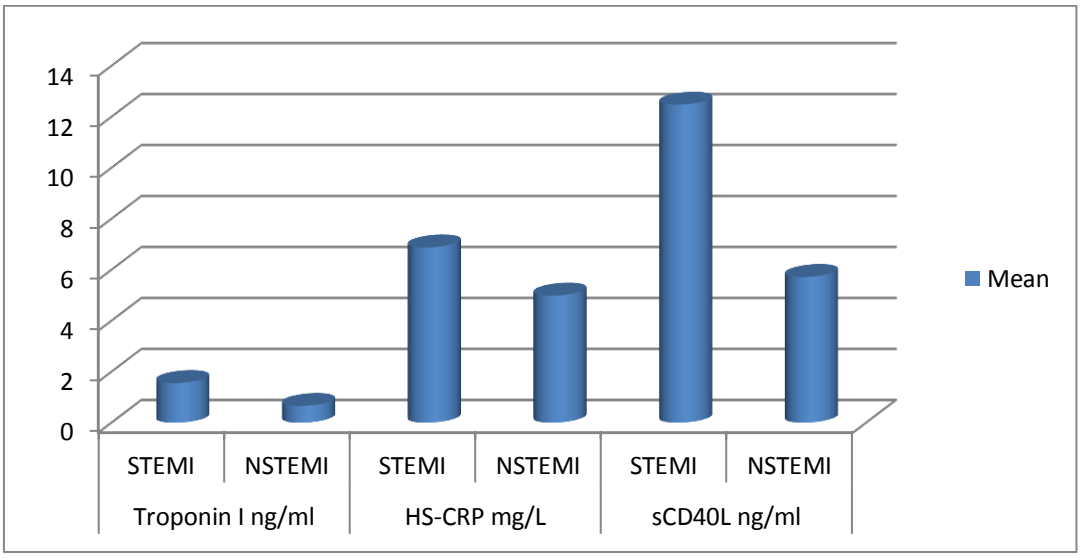

Fig (22): Comparison between patients with STEMI versus those with NSTEMI as regards Troponin I, HS-CRP and sCD40L.

Table (14): Correlation between sCD40L and age, CK-MB & Troponin I among the patient group.

		Pearson Correlation	P value	Significance
sCD40L (mg/ml)	Age (years)	0.245	0.057	NS
	CK-MB (U/L)	0.328	0.01	S
	Troponin I (ng/ml)	0.359	0.005	HS

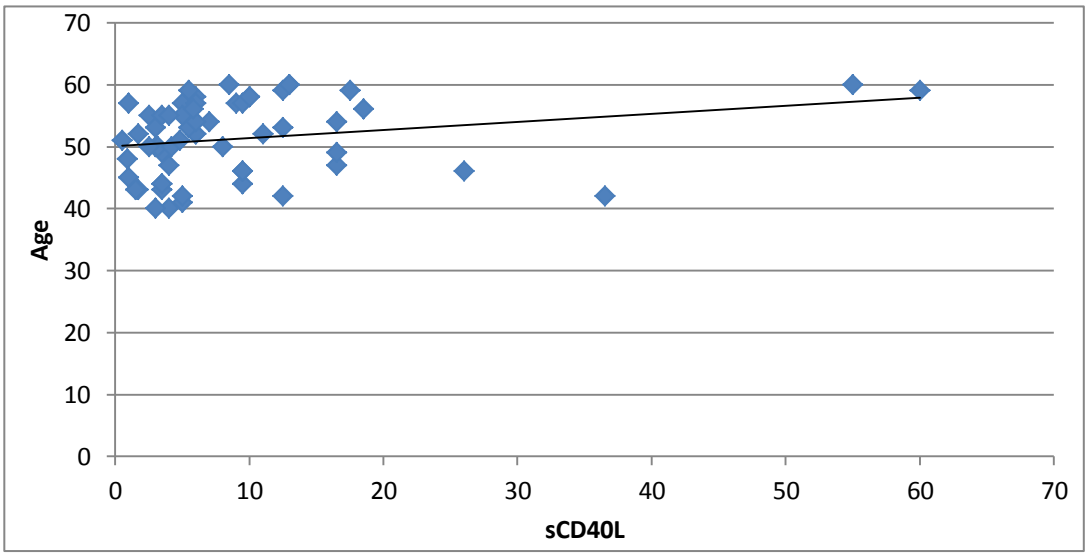

Figure (23): Correlation between sCD40L (ng/ml) and age (years).

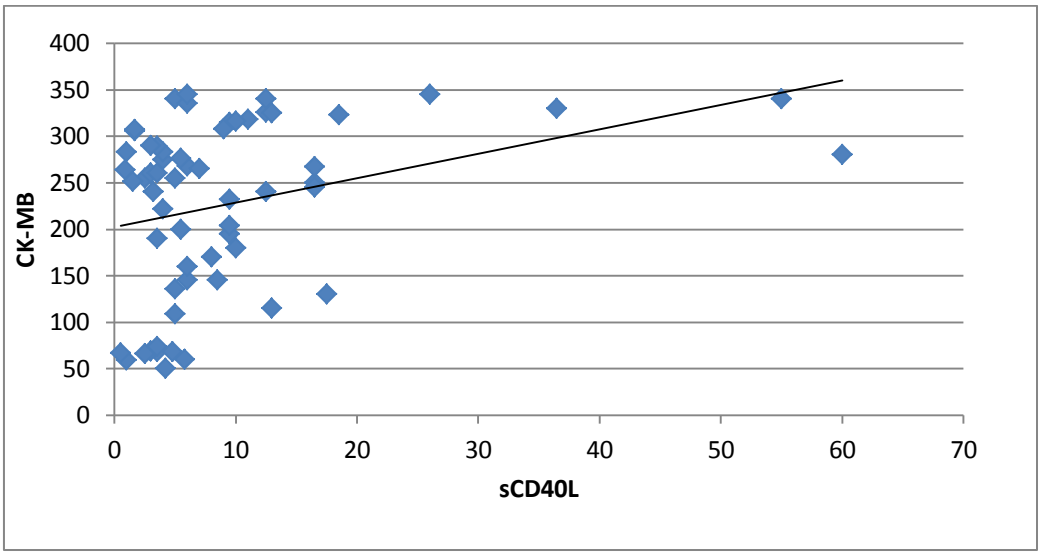

Figure (24): Correlation between sCD40L (ng/ml) and CK-MB (U/L)

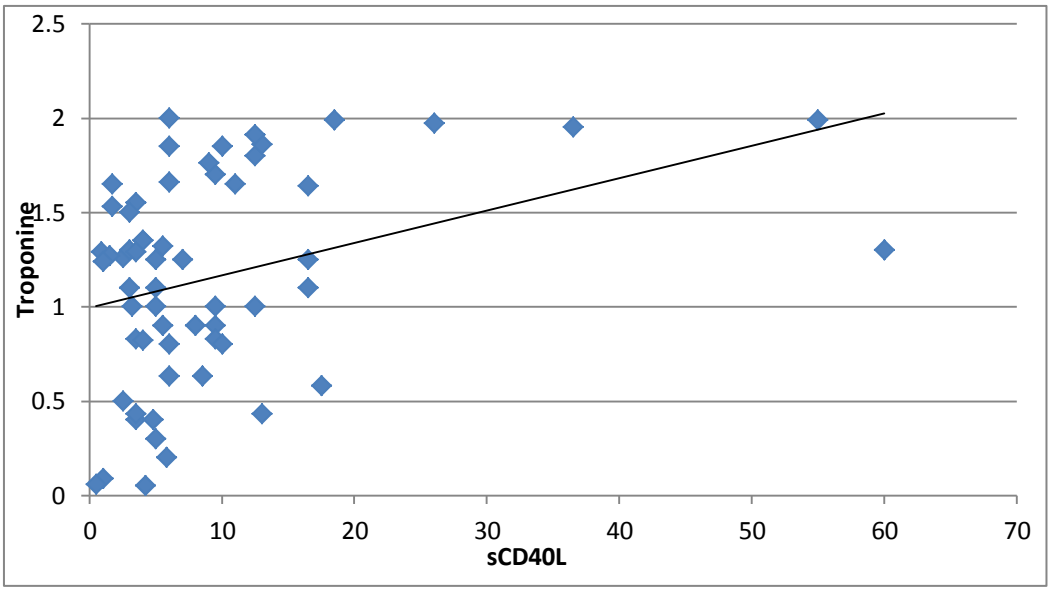

Figure (25): Correlation between sCD40L (ng/ml) and Troponin I (ng/ml).

- There is no correlation between sCD40L and age.
- There is a significant positive correlation between sCD40L and CK-MB.
- There is a highly significant positive correlation between sCD40L and Troponin I.

Table (15): Correlation between HS-CRP and CK-MB & Troponin I among the patient group.

		Pearson Correlation	P value	Significance
HS-CRP (mg/L)	CK-MB (U/L)	0.404	0.001	HS
	Troponin I (ng/ml)	0.347	0.007	HS

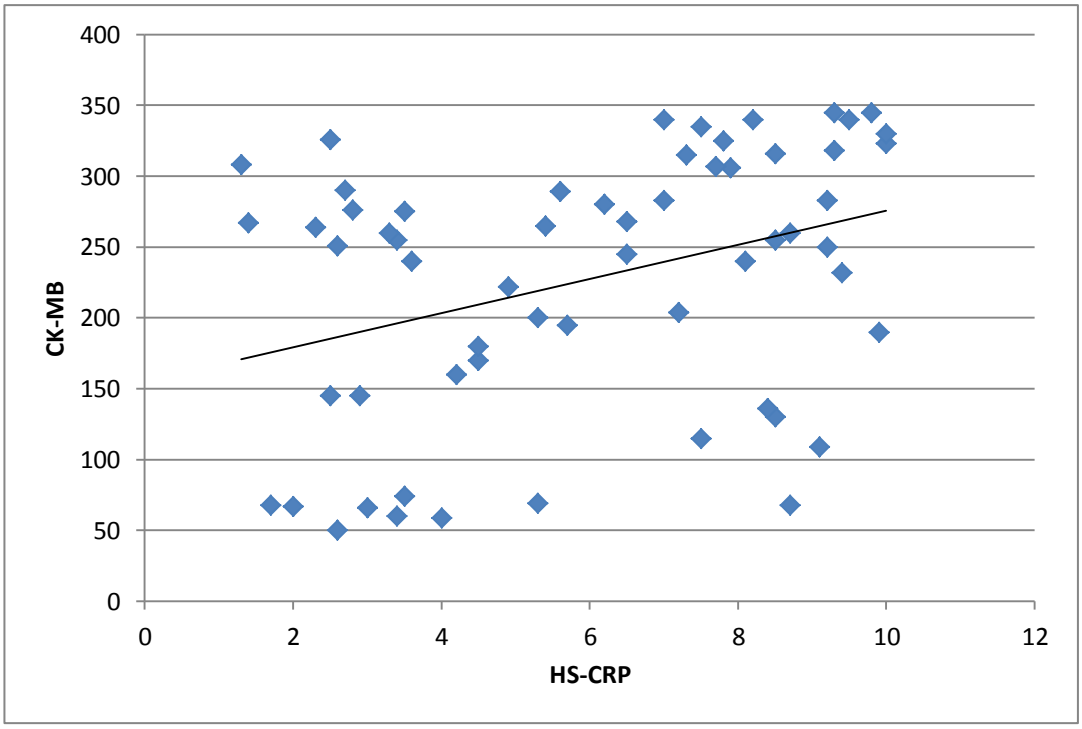

Figure (26): Correlation between HS-CRP (mg/L) and CK-MB (U/L).

- There is a highly significant positive correlation between HS-CRP and both CK-MB and troponin I.

Table (16): Correlation between sCD40L & HS-CRP.

	Pearson correlation	P value	Significance
sCD40L (ng/ml) / **HS-CRP (mg/L)**	0.320	0.012	S

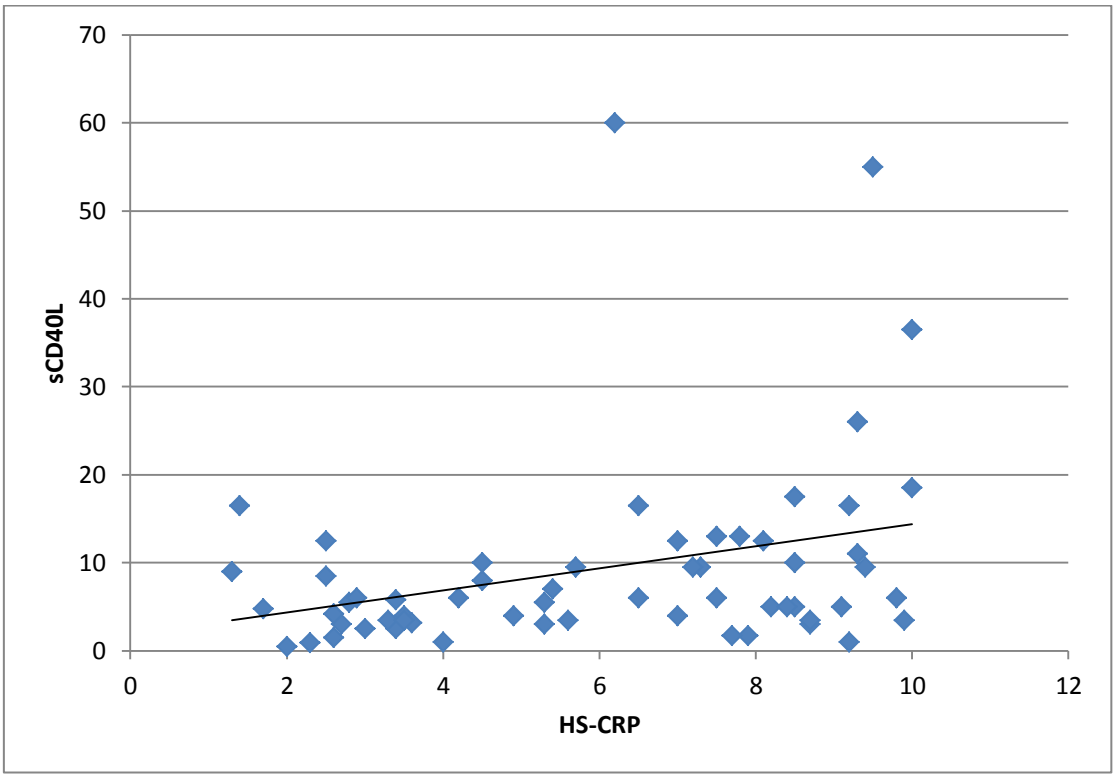

Figure (27): Correlation between HS-CRP (mg/L) and sCD40L (ng/ml).

❖ There is a significant positive correlation between HS-CRP and sCD40L.

Table (17): Predictors for high sCD40L level.

Variable	Odds ratio	95 % CI	P value
Smoking status	2.75	0.934-8.1	0.043 S
Type Of MI	1.074	0.716-1.611	0.729 NS

Multiple stepwise regression analysis was done using elevated sCD40L level as dependent factor and smoking status, type of MI as independent factors. It was found that smoking status is a significant independent predictor for elevation of the sCD40L level.

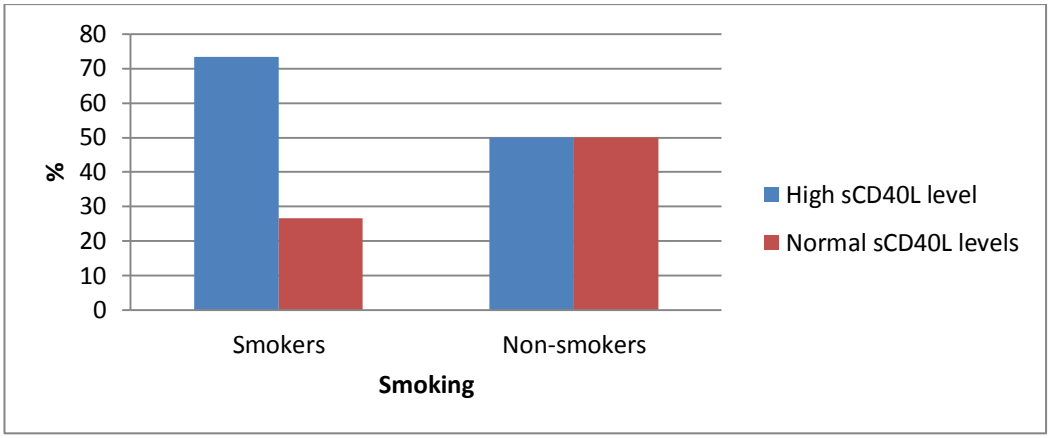

Fig (28): Smoking as independent predictor for elevation of the sCD40L level.

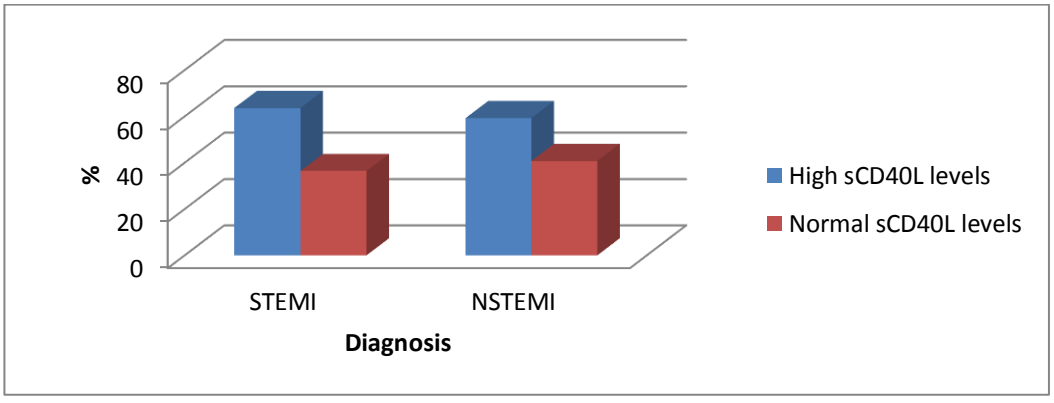

Fig (29): Type of MI as independent predictor for elevation of the sCD40L level.

Table (18): Predictors for high HS-CRP level.

Variable	Odds ratio	95 % CI	P value
Smoking status	2.95	0.998-11.139	0.035 S
Type Of MI	1.5	0.638-3.527	0.342 NS

Multiple stepwise regression analysis was done using elevated HS-CRP level as dependent factor and smoking status, type of MI as independent factors. It was found that smoking status is a significant independent predictor for elevation of the HS-CRP level.

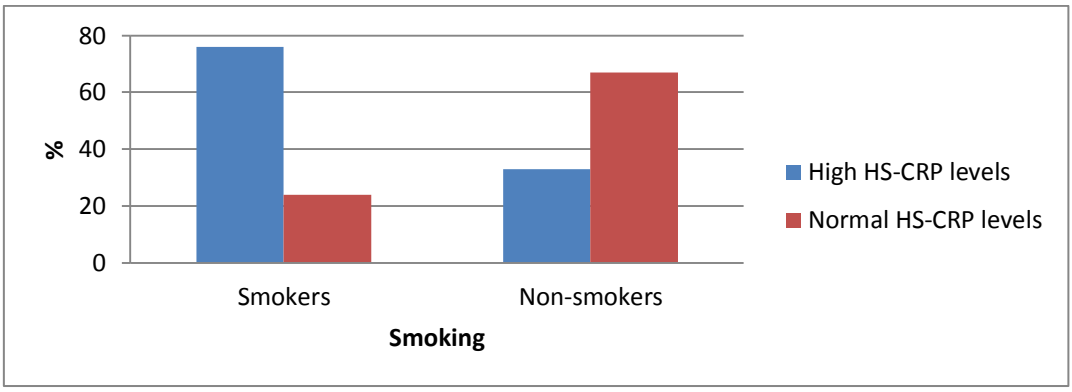

Fig (30): Smoking as independent predictor for elevation of the HS-CRP level.

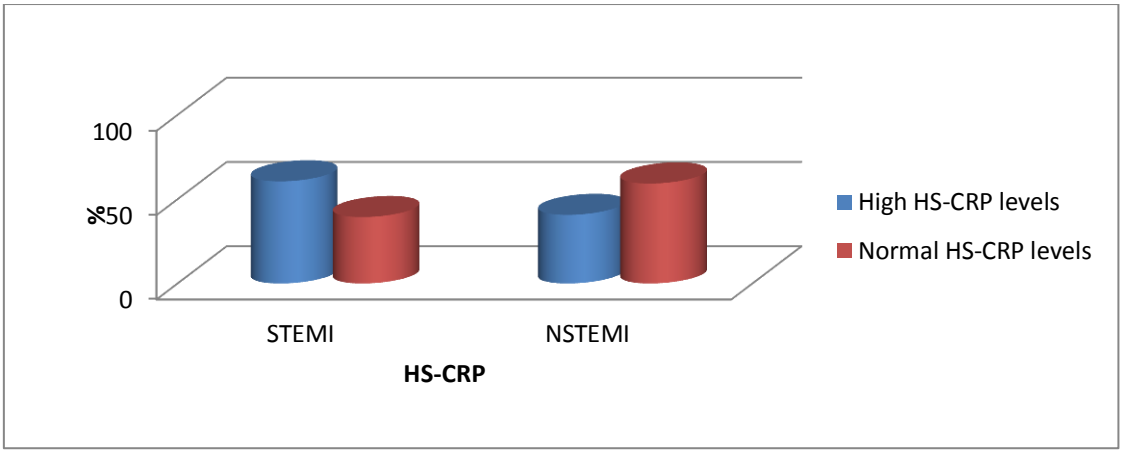

Fig (31): Type of MI as independent predictor for elevation of the HS-CRP level.

Discussion

CD40 ligand (CD40L; CD154) is a homotrimeric type II transmembrane protein. Its C-terminus contains a tumor necrosis factor homology domain required for binding to its receptor CD40. Intracellular CD40L is expressed on platelet membranes in response to platelet activation. Subsequent cleavage by metalloproteases produces soluble CD40L (sCD40L), an 18-kDa soluble fragment ***(Henn et al., 2001).***

Many reports have suggested that sCD40L is a promising clinical biomarker of atherothrombotic risk ***(Lutgens et al., 1999).*** Increased concentrations of sCD40L were reported mostly in disorders associated with platelet activation such as acute and stable coronary artery disease ***(Marx et al., 2003).*** In addition, several studies demonstrated that the circulating level of CD40L is related to a high thrombus burden in acute MI and long-term mortality***(Andre et al.,2002a).***Cigarette smokers also have upregulation of the CD40/CD40L dyad***(Kayrak et al., 2011).***However, there is no enough reports about circulating levels of CD40L in smokers with acute MI.

Myocardial infarction (MI) can be defined from a number of different perspectives related to clinical, electrocardio-graphic(ECG), biochemical and pathologic characteristics. The term MI also has social and psychological implications, both as an indicator of a major health problem and as a measure of disease prevalence in population statistics and outcomes of clinical trials***(Alpert et al.,2000).***

Cigarette smoking is accepted as one of the major risk factors that increase the risk of coronary artery disease. A large number of young people suffering from MI are smokers. There are several ways in which tobacco can influence the cardiovascular system. Smoking accelerates atherosclerosis through damage to

the endothelium and is associated with proinflammatory and prothrombotic responses. In vivo platelet activation occurs immediately after smoking a cigarette ***(Kayrak et al., 2011).***

The present study was designed to evaluate levels of sCD40L in smokers with acute MI versus non-smokers to identify the subgroup of patients who are likely to benefit from treatment with the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonists.

The current work was conducted on 79 subjects subdivided into two groups. Patient group which included 60 patients with acute myocardial infarction and control group included 19 apparently healthy individuals of matched age and sex. Patients with diabetes mellitus, dyslipidaemia, atrial fibrillation, renal or hepatic failure, significant valvular abnormalities, chronic obstructive pulmonary disease, active inflammatory or connective tissue diseases, malignancy, febrile disorders, cardiogenic shock patients requiring intra-aortic balloon pump therapy, patients with a history of recent surgery or trauma within the preceding 2 months were excluded.

All patients were subjected to full history taking and clinical examination. Blood samples were drawn from all subjects to assess levels of FBS, Total Cholesterol, TG, HDL-Cholesterol, LDL-Cholesterol, Urea, Creatinine, ALT, AST, Albumin, Total Bilirubin, PT, Troponin I, CK-MB, HS-CRP and sCD40L.

Either one of the following criteria satisfies the diagnosis of acute, evolving or recent MI: Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following: a) ischemic symptoms; b) development of pathologic Q waves on the ECG; c) ECG changes indicative of ischemia (ST segment elevation or depression); or d) coronary artery intervention (e.g., coronary angio-plasty)***(Alpert et al.,2000).***

The present study showed a statistically highly significant increase in Troponin I & CK-MB mean levels in patients with AMI versus control group. These results were in agreement with ***Pasupathi et al. (2009)*** who reported that CK, CK-MB, Troponin T and Troponin I levels were significantly increased ($p<0.001$) in subjects suffering from MI and IHD compared with control subjects.

Also, ***Garlichs et al. (2001a)*** reported that patients with unstable angina and AMI had significantly higher concentrations of Troponin I and CK-MB than patient with stable angina or controls ($p< 0.001$).***Kasap et al. (2007)*** found that Serum TnT and CK-MB levels were also significantly high in AMI patients as compared to controls ($p<0.01$; $p<0.01$, respectively).

Possible explanation for elevated levels of CK-MB and Troponin I is myocardial necrosis ***(Yilmaz et al., 2006).*** CK-MB normally exists in the cellular compartment and leak out into the plasma during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum. Troponins I is protein of the troponin regulatory complex involved in cardiac contractility. It has very high myocardial tissue specificity, are not detectable in the blood of healthy persons and offers an improved sensitivity and specificity for AMI versus a combination of ECG and traditional biochemical markers ***(Kasap et al., 2007).***

A subgroup analysis in the present study revealed that CK-MB and Troponin I levels were significantly higher in smokers as compared to non-smokers in the MI group. These results were in consistence with ***Pasupathi et al. (2009)*** who studied the effect of cigarette smoking on lipids and oxidative stress biomarkers in patients with IHD and AMI, and found that the level of myocardial injury markers were significantly higher in smokers as compared to non-smokers in the MI group. They explained that by results found on the

atherogenic effects of smoking that are mediated in part by free radical damage to lipids and possible breakdown of antioxidant status.

Most of the registry data on AMI have enrolled patients with STEMI, whereas data from patients with NSTEMI are analyzed separately with unstable angina. All have similar pathophysiology, although they differ in the degree of vessel obstruction and the extent of myocardial necrosis. They are combined into a spectrum of clinical syndromes named acute coronary syndromes. The recognition of STEMI remains important because it generally mandates the need for emergent perfusion ***(Thygesen et al., 2012).***

In the present study comparative statistics registered a statistically significant increase in CK-MB and Troponin I levels in STEMI subgroup compared to NSTEMI subgroup.

The degree of coronary obstruction may affect degree of myocyte injury and hence Troponin I and CK-MB levels. An occlusive thrombus in the absence of significant collateral vessels most often results in STEMI. ***Diderholm et al. (2002)*** stated that angiographic studies had shown that evidence of thrombus, complex lesions and reduced TIMI (Thrombolysis In Myocardial Infarction) flow grade were more common in patients with elevated troponin levels than in those with normal levels.

On comparing sCD40L levels among the studied group, a statistically significant increase was found in patient group versus control group. A subgroup analysis showed a statistically significant increase in sCD40L levels in smoker subgroup versus non-smoker subgroup in MI patients. On comparing the patients subgroups as regarding admission diagnosis, patients with STEMI had significantly higher sCD40L levels than those with NSTEMI.

In concordance with these findings, ***Garlichs et al. (2001a)*** reported raised concentrations of sCD40L in patients with unstable angina and to a greater extent those with MI ($p< 0.001$), compared with the other study groups (control and unstable angina). Also, ***Tan et al. (2008)*** revealed a statistically significant increase in sCD40L levels in patients with STEMI versus control group ($p<0.001$).

Antoniades et al. (2006) evaluated the effect of genetic polymorphisms C807T & G1648A of platelet glycoprotein Ia on the release of sCD40L during the acute phase of MI and stated that serum levels of sCD40L were significantly increased during the acute phase of MI.

Also, ***Heeschen et al. (2003)*** demonstrated that the levels of sCD40L were significantly higher in patients with acute coronary syndrome (62% of the patient with STEMI) than in patients with stable angina and patients without heart disease.

The subgroup analysis (according to the smoking status) results come near to the results obtained in a cross-sectional study done by ***Kayrak et al. (2011)***. They included a total of 57 patients with AMI. Thirty one smokers were compared with 26 non-smokers. The circulating levels of sCD40L were higher in smokers than in non-smokers ($p< 0.01$). In parallel to this finding, ***Plaikner et al. (2009)*** in subgroup analysis of patients who had a well-documented smoking status revealed that smoking was significantly associated with higher sCD40L concentrations ($p < 0.006$).

There are limited data about the different levels of sCD40L in patients with STEMI versus those with NSTEMI. ***Gururajan et al. (2009)*** documented increased levels of sCD40L in patients with STEMI versus those with NSTEMI.

Also ***Aukrust et al. (2004)*** noted that patients with stable and unstable angina had elevated levels of sCD40L compared with controls; particularly high levels occurred in patients with unstable disease indicating a gradual increase in sCD40L levels with ACS progression.

These findings could be explained by the fact that sCD40L has an unfavorable effect on the vascular redox state and endothelium-dependent relaxation, through activation of specific redox-sensitive intracellular pathways ***(Erbel et al., 2007).***

In addition, interactions between CD40L and CD40 also affect the release of adipocytokines from adipocytes, contributing to atherogenesis ***(Poggi et al., 2009).*** Evidence also suggests that CD40/CD40L interactions induce the expression of matrix metalloproteinases that degrade interstitial collagen and the thin fibrous cap of atheromatous plaques, leading to plaque instability and rupture ***(Antoniades et al., 2009).***

In addition to atheroma formation and plaque weakening, CD40/CD40L interactions have an eminent role in thrombotic events after plaque rupture. The CD40/CD40L interactions induce tissue factor expression on macrophages and ECs and diminish thrombomodulin expression, favoring a local procoagulant and prothrombotic status ***(Aukrust et al., 1999***).

Beside the interaction between CD40L and CD40, ***Zirlik et al. (2007)*** demonstrated that sCD40L also interacts with the monocyte/macrophage integrin Mac-1, resulting in Mac-1–dependent adhesion and migration of inflammatory cells ***(Zirlik et al., 2007).***

At the same time, these findings may provide evidence supporting a pathogenic link between sCD40L and ACS diagnosis as well as risk stratification***. Varo et al. (2003)*** reported that elevated levels of sCD40L identify

patients with acute coronary syndrome at higher risk of death and recurrent MI independent of other predictive variables, including cTnI and CRP.

Gururajan et al. (2009) recommended that initial estimation of sCD40L which signals inflammation, followed by serial testing of troponin can efficiently augment the diagnosis of acute coronary syndromes. They also found that multivariate logistic regression analysis revealed that sCD40L levels were highly significant on comparison with gold standards. Out of total study population (485), 297 subjects were diagnosed as ACS, of which sCD40L was positive in 267 samples on comparison with troponin (161) and CK-MB (169).

Smokers may have higher sCD40L values due to the more thrombogenic composition of the infarct lesion in smokers. Smoking enhances platelet aggregation, activation and then thrombus formation ***(Leone, 2007)***. sCD40L is released in large amounts from platelets on activation. sCD40L released from platelets upon activation indicates the hypercoagulable state promoting vascular thrombosis in active smokers ***(Aukrust et al., 1999).***

Harding et al. (2004) was the first to demonstrate that cigarette smoking is associated with upregulation of the CD40/CD40Ldyad on monocytes ($p< 0.006$), platelets ($p < 0.03$) and also associated with increased platelet-monocyte aggregation. These findings provide an important link between smoking and the development of atherothrombosis.

Youssef et al. (2007) studied the relation between the circulating level of sCD40L and thrombus burden in the infarct-related artery. In the acute phase of MI, sCD40L levels were significantly higher in the higher thrombus forming group compared to the low thrombus forming group. This could justify the results of the present study that patients with STEMI had higher sCD40L levels.

High sensitive C-reactive protein, a member of the pentraxin family of proteins, is an acute-phase reactant, increasing in response to infection, ischemia, trauma, burns, and inflammatory conditions. Although primarily synthesized in the liver, there is evidence for local expression of CRP in macrophages of the lung and the brain. In atherosclerotic plaques, it has been found associated with complement proteins and within foam cells (***Burke et al., 2002***).

A growing number of studies suggest that HS-CRP is an independent risk factor for atherosclerotic vascular disease. The baseline plasma concentration of HS-CRP predicts the risk of future myocardial infarction and stroke and is associated with a poor prognosis in unstable angina. Elevations of HS-CRP in acute coronary syndromes highlight the importance of inflammation in atherosclerotic lesions ***(Scirica et al., 2007).***

The results of the present study showed a statistically significant increase in HS- CRP levels comparing patient group versus control group. As regarding subgroup analysis, it revealed a statistically significant increase in HS-CRP levels in patients with STEMI versus those with NSTEMI. The subgroup analysis also noted that concentrations of HS-CRP were higher in smokers.

The idea that HS-CRP is elevated in patients with MI was previously addressed by ***Scirica et al. (2007)*** **in the TIMI study group** (which is one of the largest studies to investigate early determination of HS-CRP in MI), who concluded that there is a statistically significant increase in HS-CRP concentrations in patients with ACS versus control group ($p< 0.001$).

In addition, ***Honarmand et al. (2011)*** who studied HS-CRP level in 100 MI patients & 100 controls reported a statistically significant increase in HS-CRP concentrations in the patient group versus control group ($p< 0.002$). ***Martins et al. (2006)*** compared MI group with another group with no CAD and

recorded statistically significant increases in the levels of the inflammatory markers (with HS-CRP as one of them) in the MI group versus the other group ($p< 0.001$).

Considering the admission diagnosis**, *Scirica and colleages (2007)*** found that the mean concentrations of HS-CRP were significantly higher in patients with STEMI versus those with NSTEMI ($p< 0.001$)**.**

Similarly, ***Madadi et al. (2013)*** studied HS-CRP levels in 30 patients with MI and 30 patients with unstable angina and found a statistically significant increase in MI patients and recommended 3.27 mg/L as a cut-off point for diagnosis of MI.

The findings of the present study that smokers had higher HS-CRP levels were more or less similar to those found by ***Scirica et al. (2007).*** They noted that HS-CRP concentrations were statistically increased in smokers versus non-smokers.

This comes near to the results done by ***Burke et al., 2002*** who collected postmortem sera from 302 autopsies of men and women without inflammatory conditions other than atherosclerosis and assayed them for HS-CRP. There were 73 sudden deaths attributable to atherothrombi, 71 sudden coronary deaths with stable plaque, and 158 control cases (unnatural sudden deaths and noncardiac natural deaths without conditions known to elevate CRP). The HS-CRP concentrations were higher in smokers versus non-smokers ($P= 0.03$).

Recent investigations of atherosclerosis focused on inflammation as a key initiating event giving sufficient cause for these findings. Inflammatory cytokines involved in vascular inflammation stimulate the generation of endothelial adhesion molecules, proteases and other mediators, which may enter the circulation in soluble form. These primary cytokines also induce production

of the messenger cytokine IL-6, which stimulate the liver to increase production of acute phase reactants such as CRP ***(Packard and Libby, 2008).***

Patients with STEMI may have higher values due to the greater extent of necrosis in STEMI with the subsequent inflammatory response. ***Zairis et al. (2002)*** found that higher HS-CRP levels were associated with increased MI size. ***Tanaka et al. (2005)*** reported that HS-CRP levels are associated with atherosclerotic plaque rupture, i.e. higher levels were detected in subjects with greater number of ruptured atherosclerotic plaques. ***Burke et al. (2002)*** demonstrated that there is a significant association between coronary plaque burden and HS-CRP.

Higher HS-CRP values in smokers may be attributed to the fact that smoking causes activation and release of inflammatory cells into the circulation, and an increase in circulating inflammatory mediators such as acute phase proteins and pro-inflammatory cytokines ***(Van and Hogg, 2000).***Numerous studies have shown that long-term cigarette smoking increases total WBC counts, mainly due to an increase in polymorphonuclear neutrophil(PMN) counts in the circulation of smokers***(Yarnell et al., 2000).***

These activated inflammatory cells produce a great variety of inflammatory mediators in response to cigarette smoke, first of all, acute phase proteins (APPs) and cytokines such as CRP and IL-6. Many studies have reported changes in levels of inflammatory mediators not only in the lungs but also in the circulation of healthy smokers ***(Yanbaeva et al., 2007).***

The mean levels of HS-CRP were found to be different in previous studies. In the present study the mean HS-CRP level in patients with MI was 5.98 ± 2.72 mg/l while in control group was 0.83 ± 0.33 mg/L. ***Madadi et al. (2013)*** registered the HS-CRP level to be 3.68 ± 0.68 mg/L in patients with MI with the best cutoff point for differentiating unstable angina from MI was HS-CRP

equal to or greater than 3.27 mg/L. ***Yip et al. (2004)*** recorded levels of 2.95 mg/L in patients with MI and 1.35 mg/L in subjects with unstable angina.

On the other hand, ***Honarmand et al. (2011)*** found that mean titer of HS-CRP in patients was 23.2 mg/L while it was 6.3 mg/L in control group. This might have been caused by inclusion/exclusion criteria or time elapsed since onset of acute cardiac event. The present study carefully considered over the exclusion criteria (eliminating conditions which could increase HS-CRP concentration). Also, HS-CRP starts to increase 6-12 hours after the onset of MI. So, the studies that measured HS-CRP 12 hours or more after the onset of MI mostly obtained higher values. The present study measured HS-CRP in blood samples withdrawn once the patients presented to ER department complaining of chest pain and only those confirmed to have MI were included in the study.

The present study revealed significant positive correlation between sCD40L and both CK-MB and Troponin I. There are contradictory reports regarding association between sCD40L and markers of necrosis.

These results are keeping with those obtained by ***Yan et al. (2004)*** who found that the level of sCD40L was correlated with Troponin I levels in patients with ACS ($r=0.21$, $p<0.04$) indicating that Troponins are surrogate markers for the formation of fragile thrombi. Also, ***Youssef et al. (2007)*** studied the relation between the circulating level of sCD40L and thrombus burden in the infarct-related artery. In the acute phase of MI, sCD40L levels were significantly higher in the higher thrombus formatting group compared to the low thrombus forming group.

On the other hand, ***Kayrak et al. (2011)*** showed no significant correlation between sCD40L and peak CK-MB and Troponin I levels. These differences might be attributed to the numbers and management of the selected patients. Also the methodological issues regarding the measurement of sCD40L levels

may partly explain the controversial results provided by the various clinical studies ***(Antoniades et al., 2009).***

Regarding HS-CRP levels and its correlation with CK-MB and Troponin I levels and according to the results of the present study, correlation tests have revealed a significant positive correlation between HS-CRP and both CK-MB and Troponin I levels. This goes in parallel to the finding of ***Scirica et al. (2007)*** who studied the clinical application of CRP across the spectrum of ACS for the TIMI study group. There was a significant association between concentrations of HS-CRP and peak CK-MB (r = 0.337, P< 0.001) and cardiac Troponin I (r = 0.397, P< 0.001).

This can be explained by the links between elevated HS-CRP and plaque inflammation, increased thrombosis, decrease in nitric oxide synthesis, expression of adhesion molecules, alteration of complement function and inhibition of physiological fibrinolysis and hence its link with the severity of myocardial necrosis ***(Schiele et al., 2010)***. The clinical translation of these pathophysiologic effects have been observed in randomized clinical trials, such as FRISC and TIMI 11 studies, in which patients with high HS-CRP levels had the worst clinical outcomes ***(Schiele et al., 2010)***.

The results of the present study revealed a significant positive correlation between sCD40L and HS-CRP levels. These results add to other published studies regarding this correlation. ***Plaikner et al. (2009)*** using a multiple linear regression analysis revealed sCD40L concentration to be significantly associated with HS-CRP concentration.

On pathophysiologic basis, the association between sCD40L and CRP is expected as both are inflammatory markers. CAD is the end result of atherosclerosis in which the inflammatory process likely has a central role ***(Martins et al., 2006).***

Multiple stepwise regression analysis was done using elevated sCD40L level as dependent factor and smoking status, type of MI as independent factors. It was found that smoking status is a significant independent predictor for elevation of the sCD40L level. These results confirmed the results obtained by ***Antoniades et al. (2006)*** that found that in multivariate analysis of sCD40L levels during the acute phase of AMI, smoking was an independent predictor for sCD40L levels.

A complex relationship exists between cigarette smoking, hemostatic factors and cardiovascular disease. Cigarette smoking is associated with upregulation of the CD40/CD40Ldyad and increased platelet/monocyte aggregation that was proposed as potential contributors to the atherothrombotic consequences of smoking ***(Harding et al., 2004).***

A marked reduction of flow-mediated, endothelium-dependent vasodilatation of the forearm arteries was found in long term smokers. After disruption of the endothelial layer, blood coagulation starts by platelet activation and interaction of activated platelets. Platelets also increase their activation as a response to cigarette smoke. Platelets are the source of more than 95% of circulating sCD40L ***(Kayrak et al., 2011).***

Furthermore, plasma cotinine concentrations correlated with CD40 and CD40 ligand expression, and with rate of platelet-monocyte aggregations ***(Harding et al., 2004).***

Another study suggested that oxidatively modified LDL may play the role of initial trigger for CD40/CD40L expression in human endothelial and smooth muscle cells ***(Schonbeck et al., 2002)***. Increased oxidative stress and the generation of the free oxygen radicals can result in the modification of LDL to oxidized LDL. Smokers have high oxidative stress and usually both a lower

intake and plasma level of antioxidant vitamins, which may render their LDL cholesterol more susceptible to lipid peroxidation ***(Pasupathi et al., 2009).***

Multiple stepwise regression analysis was done using elevated HS-CRP level as dependent factor and smoking status, type of MI as independent factors. It was found that smoking status is a significant independent predictor for elevation of the HS-CRP level.

Several studies have reported strong association between cigarette smoking and different APPs such as C-reactive protein (CRP) and fibrinogen ***(Frohlich et al., 2003).*** For example, the large-scale, population-based NHANES III study revealed a strong independent dose-response relationship between cigarette smoking and elevated levels of CRP and fibrinogen ***(Bazzano et al., 2003).*** This analysis was based on > 4,187 current smokers, 4,791 former smokers, and 8,375 non-smokers with smoking status based on cotinine levels. These findings are in accordance with results of another population based, cross-sectional Women's Health Study of APPs in smokers that revealed increased levels of IL-6 and HS-CRP in smoking women. ***(Bermudez et al., 2002).***

IN conclusion, this study demonstrated that circulating levels of sCD40L are increased in smokers during the early phase of acute MI. Therefore, smokers with acute MI may have an increased risk of thrombotic complications during acute MI, and therefore smokers may benefit more from optimal anti-aggregant therapy including GPIIb/IIIa receptor antagonists during the acute MI setting.

Conclusion and recommendations

Conclusion

The results of the present study in keeping with evidence from literature revealed that the circulating levels of sCD40L are increased in smokers during the early phase of AMI. Therefore, smokers with AMI may have an increased risk of thrombotic complications during AMI. Optimal anti-aggregant therapy including GPIIb/IIIa receptor antagonist may be more beneficial to patients smoking cigarettes during the acute MI setting.

Recommendations

①Results could be strengthened with additional evidence confirming activation of the sCD40L pathways.

②It is better to take serial sCD40L measurements at different intervals from the onset of AMI to determine the mean time to initial and peak elevations of sCD40L levels as well as the time it takes to return to the normal range.

③Further prospective studies measuring sCD40L in patients with stable CAD and correlating it with the risk of developing AMI should be done.

④Further prospective studies could correlate sCD40L levels during the acute MI setting with the risk of post-MI complications including mortality.

Summary

CD40 and its ligand (CD40L) are glycoproteins from the tumor necrosis factor family (***Freedman, 2003).*** The CD40–CD40 ligand system is widely distributed on a variety of leukocytic and nonleukocytic cells, including endothelial and smooth-muscle cells, (***Schonbeck et al., 2001***) and on activated platelets, (***Henn et al., 1998).***

CD40 ligand also occurs in a soluble form that is fully active biologically, termed soluble CD40 ligand which is shed from stimulated lymphocytes and is actively released after platelet stimulation ***(Henn et al., 2001)***.

Soluble CD40 ligand is proinflammatory for endothelial cells and promotes coagulation by inducing expression of tissue factor on monocytes and endothelial cells (***Urbich et al., 2001).***Moreover, soluble CD40 ligand contains a KGD (Lys-Gly-Asp) sequence, a known binding motif that is specific for the major platelet integrin *a*IIbβ3 ***(Graf et al., 1995).***

Indeed, CD40 ligand has been demonstrated to be an *a*IIbβ3 (glycoprotein IIb/IIIa) ligand and a platelet agonist and to be necessary for the stability of arterial thrombi ***(Andre et al., 2002a).***

The aim of this work was to evaluate levels of sCD40L in smokers with acute MI versus non-smokers to identify the patients who are likely to benefit from treatment with the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonists.

The present study was conducted on 79 subjects, 59 males and 20 females since April 2012 to May 2013, subdivided into two groups.

Group I: This group included 60 patients with AMI (ST segment elevation and non-ST segment elevation), including 45 males and 15

females and have been admitted to Coronary Care Unit in Benha University Hospital.

Exclusion criteria:

3- Those patients with acute MI onset > 24 h, or age > 60 years.

4- Patients with history of Percutaneous Coronary Intervention (PCI), or coronary artery bypass graft surgery.

3- Patients with diabetes mellitus, dyslipidaemia, atrial fibrillation, renal or hepatic failure, significant valvular abnormalities, chronic obstructive pulmonary disease, active inflammatory or connective tissue diseases, malignancy ,febrile disorders, cardiogenic shock patients requiring intraaortic balloon pump therapy, patients with a history of recent surgery or trauma within the preceding 2 months .

4- Patients receiving antiplatelet agents such as acetylsalicylic acid and clopidogrel for any medical reason. Also, the patients that were given these agents and heparin during transfer to the hospital were excluded.

Inclusion criteria:

3- Patients of both genders with age range (40 – 60) years with acute MI defined as having symptoms of ischemia that were verified by electrocardiography or by increased levels of biochemical markers (Creatine Kinase-MB isoenzyme [CK-MB], >25U/L [25U/L] or troponin I [TnI], >0.1 μg/L [0.1 ng/mL]) ***(Apple et al., 1995).***

4- Patients who were undergoing primary Percutaneus Coronary Intervention were included in the study.

Group II: This group included nineteen apparently healthy individuals of matched age and sex. They were 14males & 5 females

Both groups were further subdivided into 2 subgroups:

❖ A- *Smokers group* defined as those currently smoking any type of tobacco in more than ten cigarettes per day and ex-smoker patients were excluded from the study.

❖ B- *Non-smokers group*.

All individuals in the study were subjected to the following:

1- Full history taking paying attention to:

* Name, Age and date of admission.

* Risk factors:

* Smoking, number of cigarettes per day

*History of chest pain.

2- Chest pain was confirmed with E.C.G. or diagnostic coronary angiography findings.

3- Laboratory investigations which included:

A) Routine Laboratory Investigations:

*** Fasting blood glucose level**

*** Liver Function Tests** (Alanine Transaminase, Aspartate Transaminase, Total Bilirubin, Albumin and Prothrombin Time).

*** Kidney Function Tests** (Urea and Creatinine).

*** Lipid Profile** (Total Cholesterol, High-denisty Lipoprotein Cholesterol, Low-denisty Lipoprotein Cholesterol and Triglycerides).

B) Specific Laboratory Investigations:

*** Cardiac markers:** CK-MK and Troponin I.

*** High sensitive C-reactive protein (CRP)** using a specific enzyme-linked immunosorbent assay (ELISA) kit.

*** The concentration of soluble CD40L levels** in plasma using a specific enzyme-linked immunosorbent assay (ELISA) kit.

The results of this work were summarized as the following:

❖ There is a statistically highly significant increase in the mean sCD40L and HS-CRP concentration in patient group versus control group.

❖ There is a statistically significant increase in the mean sCD40L and HS-CRP level in smokers versus non-smokersand in patients with STEMI versus patients with NSTEMI.

❖ There is a significant positive correlation between HS-CRP and sCD40L.

❖ Multiple stepwise regression analysis was done using elevated sCD40L level as well as elevated HS-CRP as dependent factors and smoking status, type of MI as independent factors. It was found that smoking status is a significant independent predictor for elevation of both sCD40L and HS-CRP levels.

So this study demonstrated that the circulating levels of sCD40L are increased in smokers during the early phase of acute MI.

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الملخص العربي

إن البروتين سي دي – ٤٠ و رابط البروتين سي دي –٤٠ هما بروتينات سكرية (جليكوبروتينات) من عائلة عامل النخر الورمي تتواجد بشكل واسع على الخلايا البيضاء و غير البيضاء و من ضمنها الخلايا الطلائية و خلايا العضلات الملساء .

كما يتواجد رابط البروتين سي دي –٤٠أيضا في صورة ذائبة و هي نشطة بيولوجيا بالكامل تسمى رابط البروتين سي دي –٤٠ الذائب الذي يخرج من الخلايا الليمفاوية المنشطة كما يفرز بعد استحثاث الصفائح الدموية .

ان رابط البروتين سي دي –٤٠ الذائب هو مادة مساعدة للإلتهاب للخلايا الطلائية كما أنه يحفز التخثر عن طريق إظهار عامل النسيج على سطح الخلية أحادية النواة و الخلايا الطلائية . و بالأضافة إلى هذا فإنه يحتوى على تسلسل كى جي دي وهو عامل اتحاد خاص ببروتينات الانتجرين الموجودة بالصفائح الدموية (ألفا٢ بي بيتا ٣) و بالتالي فقد تم التعرف أنه محفز للصفائح الدموية و على أنه ضروري من أجل ثبات الخثرات الشريانية .

و قد هدف هذا العمل إلى تقييم مستوى رابط البروتين سي دي – ٤٠ الذائب في مرضى احتشاء عضلة القلب الحاد من المدخنين و مقارنتهم بغير المدخنين على أمل تحديد المرضى الذين يرجح أن يكونوا اكثر استفادة بالعقاقير المثبطة لمستقبلات الجليكوبروتين (٢ بي/ ٣ أ) .

و قد أجريت هذه الدرسة السريرية على79 شخص 59 من الذكور و 20 من الإناث في الفترة ما بين شهر أبريل 2012 و مايو 2013 و قد تم تقسيمهم إلى مجموعتين:

المجموعة الأولى: و شملت 60 مريضا 45 من الذكور و 15 من الاناث يعاني كل منهم من احتشاء عضلة القلب الحاد سواء ذلك المصحوب بارتفاع الشدفة اس تي أو لا و قد تم حجزهم في وحدة الرعاية القلبية المركزة بمستشفى بنها الجامعي.

معايير الاستبعاد :

- أولئك المرضى بالإحتشاء القلبي الحاد الذين مر على بداية ظهور الأعراض أكثر من ٢٤ساعة أو الذين تزيد أعمارهم عن ٦٠ سنة .
- المرضى ذووي التاريخ المرضي بطعم مجازة الشريان التاجي .
- المرضى المصابين بداء السكري ، الارتجاف الأذيني ، الفشل الكلوي أو الكبدي ، اختلال مستوى الدهون بالدم، الإختلالات الشديدة بصمامات القلب ، مرض انسداد الشعب الهوائية المزمن ، الإلتهابات و أمراض الأنسجة الرخوة ، الأورام ، أمراض الحمى ، الصدمة

قلبية المنشأ ممن يحتاجون علاج بالبالون الأبهري ، المرضى ممن أجروا عملية جراحية أو تعرضوا لإصابات في خلال الشهريين السابقين .

- إذا كان المريض يتعاطى أي مثبطات للصفائح الدموية مثل حمض الاستيل سالسيلك أو كلوبيدوجريل لأي سبب طبي ، أو اذا تم إعطاء المريض هذه العقاقير أو عقار الهيبارين في أثناء نقله إلى المستشفى

معايير الدمج :

* المرضى من الجنسين و تتراوح أعمارهم من ٤٠ إلى ٦٠ سنة ممن يعانون من إحتشاء عضلة القلب الحاد (ظهرت عليهم أعراض الإجفاء القلبي و تم التحقق منها إما برسم القلب الكهربائي أو بإرتفاع مستوى العلامات الحيوية للقلب : الكرياتين كيناز الأيزوإنزيم المرتبط بالميوجلوبين > ٦,٦ميكروجرام / مليلتر أو التروبونين أي >٣,. ميكروجرام / لتر

*شملت هذه الدراسة المرضى الذين يجرون عملية القسطرة لأول مرة .

المجموعة الثانية: وقد شملت هذه المجموعة 19 شخص من الأصحاء ظاهريا الذين لم تظهر عليهم أعراض اكلينيكية أو دلائل برسم القلب الكهربائي على وجود احتشاء عضلة القلب الحاد. و قد كانوا 14 ذكر و5 إناث.

*** وقد تم تقسيم كل من المجموعتين إلى مجموعتين فرعيتين :

- مجموعة المدخنين (يدخنون أي نوع من أنواع التبغ بمعدل أكثر من ١٠ سجائر في اليوم).
- مجموعة غير المدخنين .

و قد خضع جميع المرضى لما يلى :

التاريخ المرضى

الإسم والسن و تاريخ دخول المستشفى .

عوامل الخطورة كالتدخين و عدد السجائر في اليوم

حدوث الآلام الصدرية

الفحص الإكلينيكي و الفحوصات الأخرى :

تم التحقق من طبيعة الألام الصدرية بالفحص الإكلينيكي و رسم القلب الكهربائي و أشعة الصبغة التشخيصية على الشرايين التاجية .

الفحوصات المعملية :

الفحوصات المعملية الروتينية:

- مستوى السكر الصائم بالدم
- اختبارات وظائف الكلى (البولينا و الكرياتينين).
- اختبارات و ظائف الكبد (إنزيمات الكبد وتشمل الألنين ترانس أميناز و الأسبرتات ترانس أميناز و الفوسفاتاز القلوي و كذلك نسبة الألبومين و نسبة البليروبين الكلية و زمن البروثرومبين).
- نسبة الدهون بالدم (الكوليستيرول و الدهون الثلاثية و كوليستيرول الليبوبروتين عالي الكثافة و كوليستيرول الليبوبروتين منخفض الكثافة).

الفحوصات المعملية الخاصة:

- كرياتين كيناز-إم بي و التروبونين أي
- البروتين التفاعلي سي – عالي الحساسية (سي آر بي – عالي الحساسية)
- مستوى رابط البروتين سي دي 40 الذائب في بلازما الدم عن طريق استخدام الفحص المناعي المتعلق بالإنزيم (إليزا)

- و قد أظهرت نتائج هذه الدراسة وجود ارتفاعا ذو دلالة احصائية في متوسط تركيز رابط البروتين سي دي 40 الذائب و كذلك البرووتين التفاعلي سي – عالي الحساسية في مجموعة المرضى مقارنة بمجموعة الاصحاء. و كذلك أظهر التحليل الإحصائي للمجموعات الفرعية للمرضى وجود ارتفاعا ذو دلالة احصائية في مستوى رابط البروتين سي دي 40 الذائب و كذلك البرووتين التفاعلي سي – عالي الحساسية في مجموعة المدخنين و في مجموعة المرضى الذين يعانون من احتشاء عضلة القلب الحاد المصحوب بارتفاع الشدفة اس تي .

- كما أظهرت النتائج وجود علاقة ذو دلالة احصائية ايجابية بين مستوى رابط البروتين سي دي 40 الذائب و مستوى البرووتين التفاعلي سي – عالي الحساسية.

- و عند استخدام نموذج الإنحدار الخطي المتعدد لاختبار التنبؤ بارتفاع مستوى كلا من رابط البروتين سي دي 40 الذائب والبرووتين التفاعلي سي – عالي الحساسية و

باستخدام كلا من التدخين و نوع احتشاء عضلة القلب الحاد فقد وجد أن التدخين هو عامل تنبؤ ذو قيمة احصائية لارتفاع مستوى كلا من رابـــط البـــروتين ســـي دي 40 الـــذائب والبرووتين التفاعلي سي – عالي

و بالتالي فقد أظهرت هذه الدراسة ارتفاع مستوى رابط البروتين سي دي 40 الذائب في المدخنين مـــن مرضى احتشاء عضلة القلب الحاد ، و بالتالى فانهم قد يكونوا أكثر عرضة للإصـــابة بالمضـــاعفات الناتجة عن الجلطات. وبالتالي فانه من المرجح أن يكونوا اكثر استفادة بالعقاقير المثبطـــة لمســـتقبلات الجليكوبروتين (٢ بي/ ٣ أ) في أثناء احتشاء عضلة القلب الحاد.

قياس مستوى رابط البروتين سي دي - ٤٠ الذائب في البلازما لدى المدخنين من مرضى احتشاء عضلة القلب الحاد

بحث مقدم من الطبيب

أميرة أسامة عبد الغفار

بكالوريوس الطب و الجراحة – كلية الطب جامعة بنها

توطئة للحصول على درجة الماجيستير فى الباثولوجيا الإكلينيكية و الكيميائية

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كلية الطب

جامعة بنها

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