INTRODUCTION

I. Diabetes mellitus:

Diabetes mellitus (DM) is a complex metabolic disorder of multiple etiologies characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism that results from defects in insulin secretion, insulin action or both. [1]

Diabetes mellitus is now considered one of the main threats to human health in this century as its prevalence is in a dramatic increase, and can affect around 5 % of people all over the world. [1] It is estimated that 366 million people had diabetes mellitus in 2011; by 2030 this would have risen to 552 million. [2]

I.1. Diagnostic criteria for DM:

The diagnostic criteria for diabetes established by the World Health Organization (WHO) [3] are illustrated in **(table 1)**. The Revisions for the 2010 Clinical Practice Recommendations (CPR) now include the use of glycated hemoglobin (HbA1c) as a diagnostic criterion for diabetes, with HbA1c $\geq$ 6.5 % being diagnostic. [4]

Individuals with impaired fasting glucose (100 - 125 mg/dl) and those with impaired glucose tolerance (140 – 200 mg/dl) and with an HbA1c levels ranging between 5.7 – 6.4 % are considered prediabetic and are at considerable risk for developing diabetes mellitus as well as cardiovascular disease. [5]

Table (1): Diagnostic criteria for diabetes according to the WHO [3]

Condition	2 hours post prandial glucose (mg/dl)	Fasting glucose (mg/dl)
Normal	<140	< 110
Impaired fasting glucose	>140	$\geq$ 110 and < 126
Impaired glucose tolerance	$\geq$ 140	> 126
Diabetes mellitus	$\geq$ 200	$\geq$ 126

I.2. Classification of DM:

The following categories of DM have been described according to the American Diabetes Association (ADA). [6]

I.2.1- Type 1 DM (T1DM): is a chronic, progressive, autoimmune disorder with incomplete penetrance. In individuals with a genetic susceptibility to T1DM, an abnormal, unregulated, autoimmune response targeting β-cells can develop, which leads to progressive islet damage and insulin insufficiency. The environmental triggers that interact with susceptibility and resistance genes to initiate and perpetuate the disease remain to be defined. The incidence of T1DM in developed countries is 10–60 cases per 100,000 people, and is increasing by ~4% per year. [7] Unlike people with type 2 DM, those with

type 1 DM usually are not obese and usually present initially with diabetic ketoacidosis (DKA). The distinguishing characteristic of a patient with type 1 DM is that if his or her insulin is withdrawn, ketosis and eventually ketoacidosis develop. Therefore, these patients are dependent on exogenous insulin. [8]

Type 1 DM can occur at any age. It is most common in juveniles but can also develop in adults. It is well known that autoimmune destruction of β-pancreatic cells characterizes T1DM with presence of T cells reactivity to islets antigens and circulating autoantibodies to glutamic acid decarboxylase 65 (GADA65), islet cell cytoplasm (ICA), tyrosine phosphatase like protein IA-2A and insulin (IAA). [9, 10] The absence of phenotypic features of T1DM is taken as indication of T2DM. However, there is another subset of adult patients are initially categorized as T2DM phenotype but were seropositive for autoantibodies a hallmark of β-cells destruction accounting for 2% - 12% of all cases of diabetes termed as latent autoimmune diabetes of adults (LADA) with alternative names as 1.5 diabetes, latent type 1 diabetes, slowly progressive insulin dependent diabetes, youth onset diabetes of maturity. [11]

LADA patients are typically diagnosed after 35 years of age and have initial good glycemic control with sulfonylureas but eventually become insulin dependent more rapidly than T2DM patients. Due to latent nature, LADA patients are often misdiagnosed as T2DM. [12] Internationally, rates of type 1 DM are increasing. In Europe, the Middle East, and Australia, rates of type 1 DM are increasing by 2-5% per year.[13]

I.2.2- Type 2 diabetes mellitus (T2DM): also called noninsulin-dependent diabetes mellitus. (will be mentioned later)

I.2.3- Gestational diabetes mellitus (GDM): is defined by the American Diabetes Association as "glucose intolerance of any degree with onset or first recognition during pregnancy". Diagnostic criterion of two hours plasma glucose more than 140 mg/dl after 75 gram oral glucose load is a modified version of WHO guidelines in that WHO procedure requires women to be in basal fasting state. [14]. It occurs in about 2–5 % of all pregnancies and may improve or disappear after delivery. In addition, about 20–50 % of affected women develop type 2 diabetes later in life. [15]

Furthermore, incidence rates of GDM are increasing, which could reflect the increased prevalence of obesity and T2DM within the general population. Both obesity and a family history of T2DM represent important risk factors for the development of GDM.[16]

I.2.4- Type 3 diabetes mellitus (T3DM): Alzheimer's disease is considered by some investigators as type 3 diabetes mellitus because of impaired glucose uptake in the central nervous system. [17]

I.2.5- Diabetes mellitus of other causes: this may include DM caused by genetic impairment of pancreatic β-cells or abnormal coding of insulin release and action, drug- and toxin-induced DM, and other types that may not fit into the previous four categories. It has been shown that patients with mutations in insulin receptors may also develop a variety of other conditions including polycystic ovarian disease and acanthosis nigricans. [18]

II. Type 2 diabetes mellitus (T2DM):

II.1. Definition:

T2DM consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion and excessive or inappropriate glucagon secretion. [19]

Unlike patients with type 1 diabetes mellitus, patients with type 2 are not absolutely dependent on insulin for life. This distinction was the basis for the older terms for types 1 and 2, insulin dependent and non–insulin dependent diabetes. However, many patients with type 2 diabetes are ultimately treated with insulin. Because they retain the ability to secrete some endogenous insulin, they are considered to require insulin but not to depend on insulin. Nevertheless, given the potential for confusion due to classification based on treatment rather than etiology, the older terms have been abandoned. [19]

Another older term for type 2 diabetes mellitus was adult-onset diabetes. Currently, because of the epidemic of obesity and inactivity in children, type 2 diabetes mellitus is occurring at younger ages. Although type 2 diabetes mellitus typically affects individuals older than 40 years, it has been diagnosed in children as young as ten years of age who have a family history of diabetes. In many communities, type 2 diabetes now outnumbers type 1 among children with newly diagnosed diabetes. [20]

II.2. Epidemiology:

The global epidemic of people with type 2 diabetes is largely due to population growth, aging, urbanization, and the scourge of obesity and physical inactivity. It is estimated that 439 million people would have T2DM by the year 2030 and it can affect about 90 % of all diabetic cases. [19, 21]

The incidence of T2DM varies substantially from one geographical region to another as a result of environmental and lifestyle risk factors. Indeed, the risk factors associated with type 2 diabetes are grouped into two categories: modifiable and non-modifiable risk factors. Modifiable risk factors include diets rich in saturated fats and simple carbohydrates, impaired glucose tolerance, metabolic syndrome, high blood pressure, elevated plasma triglycerides and low levels of physical activity. Moreover, the non-modifiable risk factors are age, family history of diabetes, ethnicity and gestational diabetes. [22, 23]

II.3. Pathophysiology of T2DM:

T2DM is one of the complicated and most prevalent type of diabetes.[24] A large number of hypotheses have been developed to describe the mechanisms, which are usually involved in the propagation of T2DM. Obesity, aging, β-cell dysfunction, tissue lipid accumulation, oxidative stress, endoplasmic reticulum stress (ER-stress) in β-cells, tissue inflammation and physical inactivity are the most commonly known factors linked to insulin resistance in T2DM. [25]

T2DM is simply characterized by uneven insulin secretion and its related effects. In terms of pathogenesis, glucolipotoxicity can be stated as one of an essential determinant of T2DM. In general, glucolipotoxicity is a common term used in combination for glucotoxicity and lipotoxicity as both are known to progress simultaneously. [26]

Multiple risk factors are involved in induction of β-cell dysfunction. These include optimal glucolipotoxicity (Hyperglycemia and dyslipidemia), which can impact the development of insulin resistance, oxidative stress and/or endothelial cells dysfunction, as well as the activation of pro-inflammatory mediators and macrophage infiltration. Collectively, these factors may lead to β-cell dysfunction due to which impairment of insulin secretion occurs that may provoke the onset of T2DM **(figure 1)**. [27]

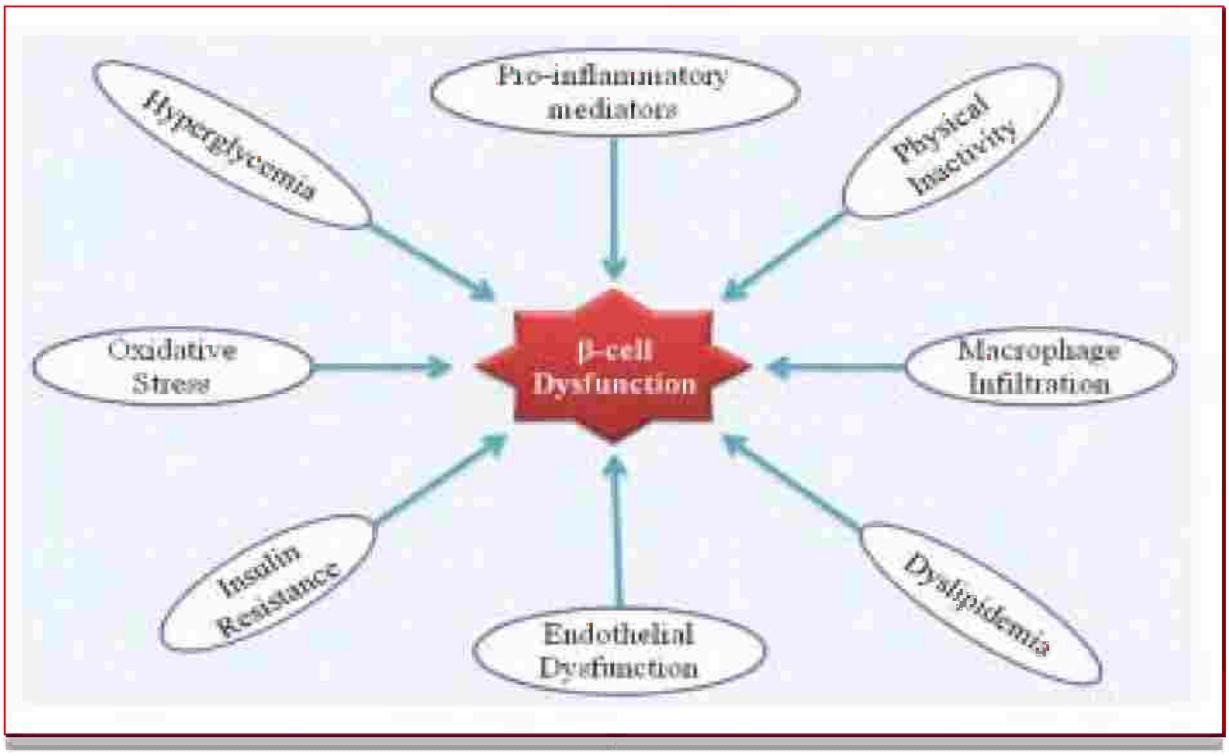

Figure (1): Mechanism of β-cell dysfunction. [27]

II.4. Inflammatory biomarkers in type 2 diabetes:

Epidemiological studies have demonstrated an increase in plasma levels of inflammatory markers such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in patients with metabolic syndrome and also in those with clinically overt T2DM. Other molecules such as the transforming growth factor (or tumor growth factor)-β1, monocyte chemoatractant protein-1 (MCP-1) or lipoprotein-associated phospholipase-A2 (Lp-PLA2) also present increased concentrations in T2DM subjects. A genetic predisposition associated to excessive caloric intake and a lack of physical exercise can lead to obesity and central adiposity. Then, this may result in adipose tissue dysfunction, macrophage infiltration and a greater release of cytokines such as IL-6 and TNF- α. [28]

The role of IL-6 in T2DM is considered to be complex and controversial, however, various experimental studies have confirmed that IL-6 induces insulin resistance in peripheral tissues, apoptosis in pancreatic islets together with other inflammatory cytokines and stimulates the inhibition of cytokine's signaling proteins. Due to these deleterious effects, IL-6 is considered as an independent risk factor and acts as predictor and pathogenic marker for insulin resistance and progression of T2DM. [29]

C-reactive protein is the principal down-stream mediator of the acute phase response and is primarily derived via IL-6-dependent hepatic biosynthesis. CRP is a member of the pentraxin family of oligomeric proteins involved with pattern recognition in innate immunity. Reported immunoregulatory functions of CRP include enhancement of leukocyte reactivity, complement fixation, modulation of platelet activation and clearance of cellular debris from sites of active inflammation. [30]

II.5. Complications of T2DM:

Indeed, chronic hyperglycemia injures the human body in different ways. One of the chief injuries arising from hyperglycemia is injury to vasculature, which is classified as either small vascular injury (microvascular diseases) or injury to the large blood vessels of the body (macrovascular diseases). [31]

II.5.1. Microvascular complications of T2DM:

II.5.1.1. Diabetic retinopathy:

Diabetic retinopathy may be the most common microvascular complication of diabetes. Its prevalence in Egypt is around 1,760,600 (20.5 %) of all diabetic cases. [32] The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia and the presence of hypertension. Most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis. Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes. [33]

II.5.1.2. Diabetic nephropathy:

Diabetic nephropathy (DN) is the leading cause of renal failure in the United States. It is defined by proteinuria of > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, called "microalbuminuria". Microalbuminuria is defined as albumin excretion of 30–299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes. [34] It is a multi-stage disorder and it takes several years to progress from the stage of incipient nephropathy through overt nephropathy to the stage of end-stage renal disease (ESRD). The earliest changes to appear with the onset of DN glomerular hyperfiltration, increased renal blood flow and hypertrophy of kidneys are potentially reversible with good glycemic control. [35]

Microalbuminuria rarely occurs with short duration of type 1 diabetes; therefore, screening in individuals with T1DM should begin after 5 years' disease duration. While, in T2DM microalbuminuria should be performed at diagnosis, Because of the difficulty in the precise dating and recommended for testing annually. Screening for microalbuminuria can

be performed by three methods: 1) measurement of the albumin-to-creatinine ratio (ACR) in a random spot collection; 2) 24-hours collection with creatinine, allowing the simultaneous measurement of creatinine clearance; and 3) timed (e.g., 4-hours or overnight) collection. The first method is often found to be the easiest to carry out in an office setting, generally provides accurate information, and is therefore preferred. Microalbuminuria is said to be present if urinary albumin excretion is ≥30 mg/24 hours (equivalent to 20 μg/min on a timed specimen or 30 mg/g creatinine on a random sample).[36, 37]

II.5.1.3. Diabetic neuropathy:

Diabetic neuropathy can be classified as peripheral, autonomic, proximal and focal. Each affects different parts of the body in various ways. Peripheral neuropathy, the most common type, causes pain or loss of feeling in the toes, feet, legs, hands and arms. While, autonomic neuropathy causes significant morbidity and even mortality in patients with diabetes, neurological dysfunction may occur in most organ systems and can be manifest by gastroparesis, constipation, diarrhea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia, and even sudden cardiac death. Cardiovascular autonomic dysfunction is associated with an increased risk of silent myocardial ischemia and mortality. In addition, proximal neuropathy causes pain in thighs, hips, buttocks and lead to weakness in the legs. Finally, focal neuropathy results in the sudden weakness of one nerve or a group nerves causing muscle weakness or pain and affects any nerve in the body. [38]

II.5.2. Macrovascular complications of T2DM:

The central pathological mechanism in macrovascular disease is the process of atherosclerosis. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from low density lipoprotein (LDL) particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles. Monocytes then infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes, which in turn induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction. [39]

In addition to atheroma formation, there is a strong evidence of increased platelet adhesion and hypercoagulability in type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in patients with diabetes. The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in type 2 diabetes. [40]

Diabetes increases the risk that an individual will develop cardiovascular disease (CVD) which considered as the primary cause of death in people with either type 1 or type 2 diabetes. [41]

Type 2 diabetes typically occurs in the setting of the metabolic syndrome, which also includes abdominal obesity, hypertension, hyperlipidemia, and increased coagulability that promote CVD. Even in this setting of multiple risk factors, type 2 diabetes acts as an independent risk factor for the development of ischemic disease, stroke, and death. Among people with type 2 diabetes, women may be at higher risk for coronary heart disease than men. The presence of microvascular disease is also a predictor of coronary heart events. Diabetes is also a strong independent predictor of risk of stroke and cerebrovascular disease, as in coronary artery disease. Patients with type 2 diabetes have a much higher risk of stroke, with an increased risk of 150–400 %. Risk of stroke-related dementia and recurrence, as well as stroke-related mortality, is elevated in patients with T2DM. [41-43]

III. Pathophysiology of diabetic dyslipidemia:

The pathogenesis of diabetic dyslipidemia is multifactorial. Insulin resistance with attendant increase in free fatty acid (FFA) flux into the liver plays a central role in promoting the typical triad of diabetic dyslipidemia, namely high plasma triglyceride concentrations, low plasma high density lipoprotein cholesterol (HDL-C), and increased concentration of small dense LDL particles. The increased flux of free fatty acids promotes hepatic triglyceride production, which in turn stimulates the secretion of apolipoprotein-B (apo-B) and very low density lipoprotein (VLDL). The VLDL-transported triglyceride is exchanged for HDL-transported cholesteryl ester through the action of the cholesteryl ester transfer protein (CETP). This exchange results in increased amounts of both atherogenic cholesterol-rich VLDL remnant particles and triglyceride rich, cholesterol-depleted HDL particles **(figure 2)**. [44] The triglyceride-enriched HDL is subsequently hydrolyzed by hepatic lipase (HL) or lipoprotein lipase (LpL), allowing the lipid-poor apolipoprotein-A1 (apo-A1) to be filtered by the renal glomeruli and degraded in renal tubular cells **(figure 2)**. [45]

Inter-particle lipid exchanges also explain the increased concentration of small dense LDL particles. Thus, CETP facilitates the transfer of triglyceride from VLDL into LDL in exchange for LDL-transported cholesteryl ester. The triglyceride-rich LDL undergoes hydrolysis by hepatic lipase or lipoprotein lipase, which results in lipid depleted small dense LDL particles (**figure 2**).[45]

Another potential mechanism for low HDL-C in diabetes includes resistance to the efficacy of insulin to upregulate the apo-A-1 production. This may contribute to low HDL-C, especially in obese individuals without hypertriglyceridemia. [46]

A third pathogenic mechanism of diabetic dyslipidemia relates to the increased production of inflammatory cytokines such as TNF-α and IL-6. These cytokines increase insulin resistance and directly downregulate apo-A1 and HDL production while increasing the activity of key enzymes promoting hypertriglyceridemia. [47]

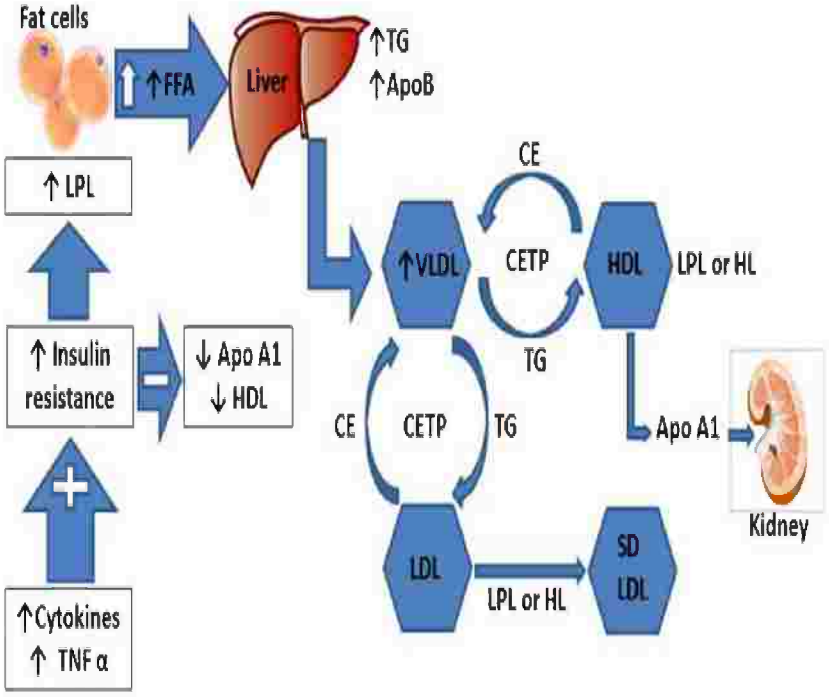

Figure (2): **Pathogenesis of diabetic dyslipidemia**. Insulin resistance initiates the characteristic triad of high triglyceride level, low HDL-C level, and high small dense LDL-C level. If the concentration of VLDL-transported triglyceride is high, CETP promotes the transfer of LDL cholesteryl ester or HDL cholesteryl ester in exchange for triglyceride. Triglyceride-rich HDL or LDL can undergo hydrolysis by hepatic lipase or lipoprotein lipase. Up arrow increased level, apo-A1: apolipoprotein-A1, apo-B: apolipoprotein-B, CE: cholesteryl ester, CETP: cholesteryl ester transfer protein, FFA: free fatty acid, HDL: high density lipoprotein, HL: hepatic lipase, LDL: low density lipoprotein, LpL: lipoprotein lipase, sd LDL: small dense LDL cholesterol, TG: triglyceride, TNF-α: tumor necrosis factor-α, VLDL: very low density lipoprotein. [45]

IV. Syndecans:

Syndecan constitute a family of transmembrane proteoglycans that perform multiple functions during development, damage repair, tumor growth, angiogenesis and neurogenesis. Through mediating binding of a great number extracelluar ligands to their receptors, these proteoglycans trigger a cascade of reactions regulating, thereby, various processes in a cell: cytoskeleton formation, proliferation, differentiation, adhesion and migration.[48]

IV.1. Structure of syndecans:

Proteoglycans that include the syndecans are produced by most animal cells. They are composed of a core protein with covalently linked glycosaminoglycan chains (GAG). The polypeptide chain of the core protein is synthesized on membrane bound ribosomes, and this is followed by transfer of the synthesized protein to the lumen of the endoplasmic reticulum. Attachment of glycosaminoglycan chains proceeds in the golgi apparatus, and

then the proteoglycans are subjected to exocytosis to be delivered to the cell surface. The polysaccharide chains are modified in the golgi apparatus by epimerization and addition of sulfate groups. Glycosaminoglycans can constitute up to 95 % of proteoglycan composition. Some proteoglycans are located only in extracelluar matrix, while others, including syndecans, are integrated into the cell membrane. [49]

There are four types of syndecans in vertebrates that, like all other proteoglycans, are composed of a core protein and covalently linked glycosaminoglycan chains. The core protein, in turn, consists of three domains: extracelluar, transmembrane, and cytoplasmic. Syndecans are located on the surface of most cell types, including fibroblasts and epithelial cells. Genes that encode syndecans are differently expressed in different tissues: the gene of syndecan-1 is expressed predominantly in epithelial tissues, whereas the gene of syndecan-2 in mesenchymal, of syndecan-3 in nervous, and of syndecan-4 in almost all tissues. Syndecans-2 and -4 are shorter than the others and contain heparan sulfate chains only, while syndecans-1 and -3 are significantly longer and contain both heparan sulfate (HS) and chondroitin sulfate (CS) chains. [50]

Both heparan sulfate and chondroitin sulfate chains are attached covalently to serine (Ser) residues, provided the next amino acid residue in the sequence is glycine (Gly). The attachment involves a "special tetrasaccharide" composed of xylose, which is successively linked to two galactose and one glucuronic acid residues. This structural unit serves as an "original" primer that initiates growth of the polysaccharide chain. Heparan sulfate consists of two repeating disaccharide units: N-acetylglucosamine glucuronic acid and N-acetylglucosamine iduronic acid, while chondroitin sulfate is composed of a repeating disaccharide unit that contains N-acetylgalactosamine and glucuronic acid **(figure 3)**. Glycosaminoglycan chains may contain from 50 up to 200 disaccharide units to which sulfate groups are attached, and this makes them negatively charged and allows sequestering a large number of positively charged molecules. Moreover, due to the action of the same charges, the glycosaminoglycan chains are repulsed from each other and are extended in space, and this increases the area of their action. [51]

Sulfation of glycosaminoglycans proceeds unequally: there are sites with a low degree of sulfation distributed among other sites with degree of sulfation. As a rule, the sparsely sulfated sites are localized closer to the protein moiety of the syndecan molecule.[51]

The cytoplasmic domain of syndecans contains conserved sites C1 and C2 that flanks the variable region (V). A stretch of four amino acid residues (EFYA) in conserved site C2 of the cytoplasmic domain binds to postsynaptic density-95/zonula occludens-1(PDZ)-binding proteins such as synbindin, synectin, CASK, and syntenin, which play the key role in vesicular transportation, synaptic signaling, neuronal migration, and metastasis of cancer. A proximal region of C1 of the cytoplasmic domain of syndecans interacts with actin binding proteins ezrin, radixin, and moesin, which control the organization of actin cytoskeleton. The variable region V is unique for each type of syndecan, and it determines their specificity. The transmembrane domain of all syndecans contains a conserved motif GxxxG that is necessary for dimerization of the syndecan and for retaining cholesterol in the membrane. [52, 53]

IV.2. Syndecans shedding:

Syndecans can exist in two forms: membrane incorporated and soluble ones. The latter form represents the ectodomain that was cleaved from the cell surface, a process performed by extracelluar zinc-dependent endopeptidases and metalloproteinases (MMP) **(figure 4)**. This cleavage is regulated by a large number of extracelluar stimulators: growth factors, chemokines, virulent components of bacteria, trypsin, heparanase, insulin, and also cellular stress. As a rule, cleavage of the ectodomain is increased in response to inflammation and many other destructive processes in an organism. Soluble syndecans-1 and -4 are accumulated in large amounts in the fluid that forms around wounds. Cleavage of the extracelluar domain proceeds more intensively under the action of thrombin and epidermal growth factor, which are especially active during wound healing. Moreover, many signaling transducers such as protein kinase-C (PKC) and nuclear transcription factor kappa-B (NF-κB) are also involved in the cleavage of the ectodomains. Phosphorylation of tyrosines in conserved sites of the cytoplasmic domain also leads to cleavage of the ectodomain. [51, 54]

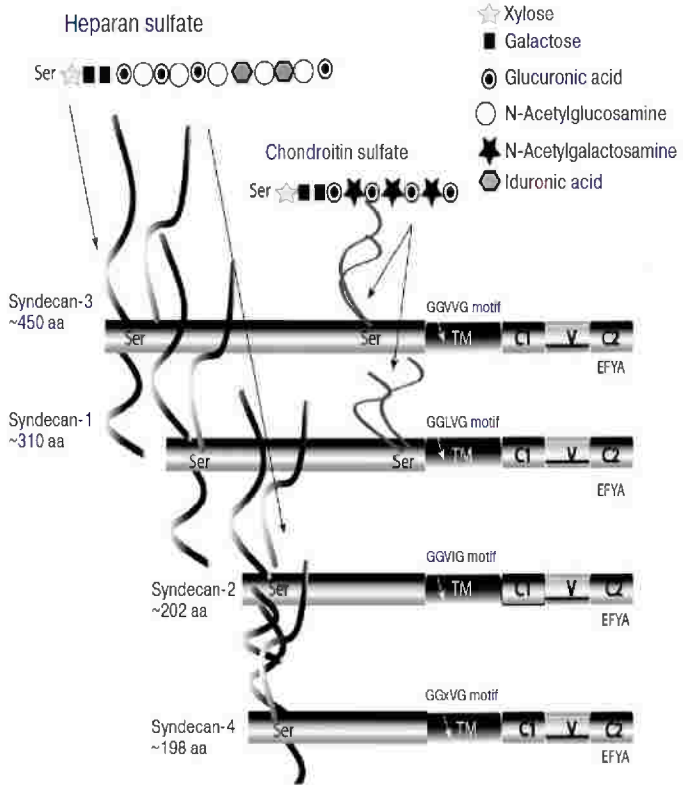

Figure (3): Schematic representation of vertebrate syndecans. Syndecans-1 and -3 are longer than syndecans -2 and -4. Glycosaminoglycan chains are covalently attached to the core protein according to a common principle: serine–xylose–two galactoses and one glucuronic acid residues. After that the repeating disaccharide units are shown: for heparan sulfate these are *N*-acetylglucosamine with glucuronic acid or *N*-acetylglucosamine with iduronic acid; for chondroitin sulfate these are *N*-acetylgalactosamine with glucuronic acid. The cytoplasmic domain contains two conserved sites C1 and C2 and one variable site V that is unique for each type of syndecan. TM, transmembrane domains containing GxxxG motifs that are conserved for each type of syndecan. [51]

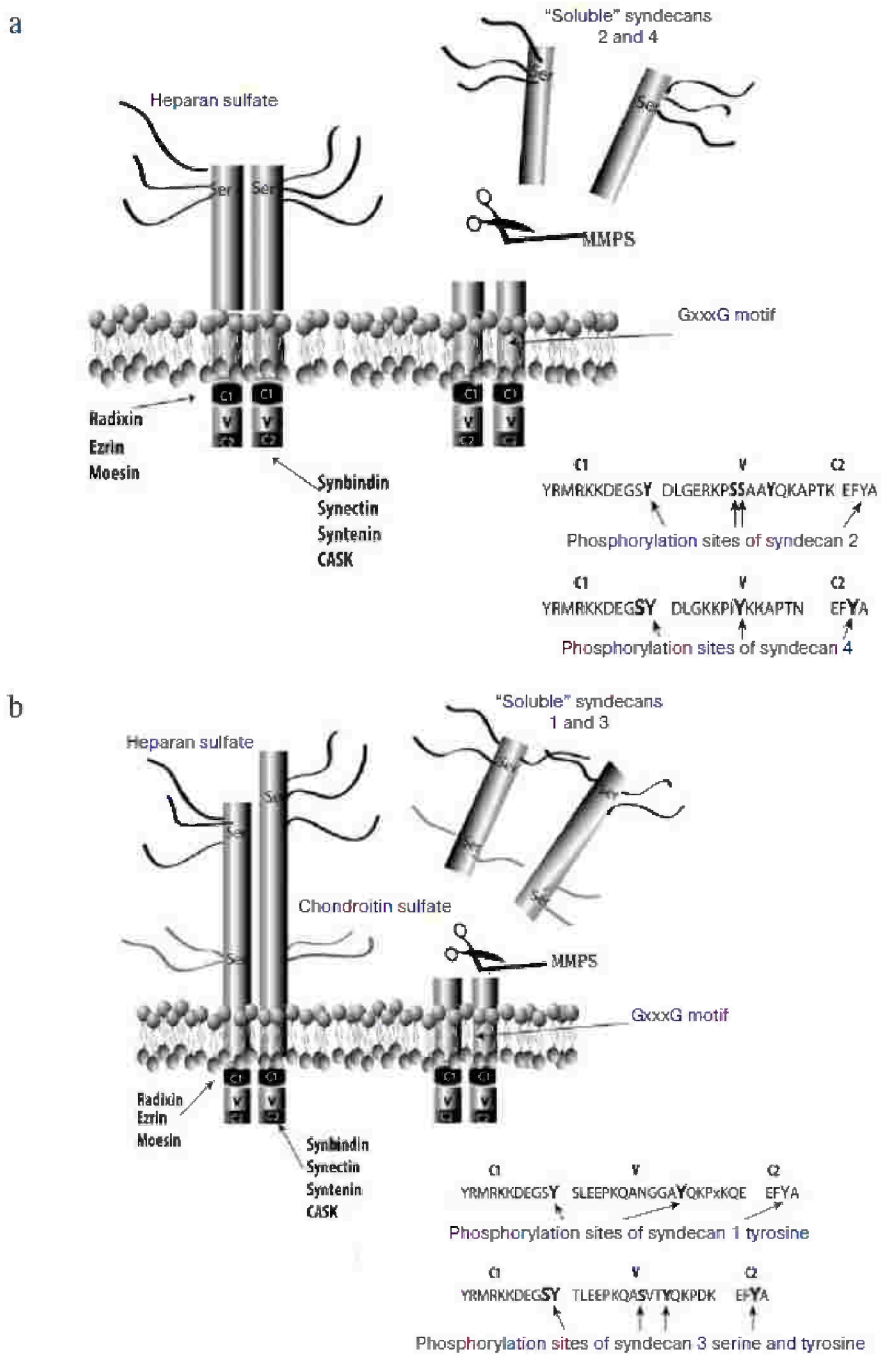

Figure (4): **Syndecans shedding:** a) syndecans 2 and 4; b) syndecans 1 and 3. The external ectodomains are cleaved from the membrane by metalloproteinases (MMPS) with formation of a "soluble" form. The cytoplasmic domain contains phosphorylation sites. The variable site in the cytoplasmic domain is unique for each syndecan. The C2 site of the cytoplasmic domain contains a PDZ-binding motif that provides contacts with intracellular adapter proteins. The C1 site interacts with intracellular actin-binding proteins. The GxxxG motif in the transmembrane domain is necessary for formation of homo- or heterodimers in the membrane. [51]

IV.3. Role of syndecan-1 in inflammation and lipid metabolism:

Syndecan-1 is associated with many physiological processes in the developing as well as the adult organism. The concentration of the soluble form of syndecan-1 in healthy organisms is relatively low. Intensive cleavage and, correspondingly, increased concentration of soluble form of syndecan-1 in the blood along with increased synthesis of syndecan-1 is characteristic for tissue repair after damage or in response to inflammation. Syndecan-1 regulates leukocyte adhesion and migration from the blood circulation to an inflammation zone. [55]

Syndecan-1 plays an important role in the absorption of intermediate density lipoproteins (IDL) and residual chylomicrons by hepatocytes. In syndecan-1 knockout mice, residual chylomicrons and IDL are accumulated. Normally, syndecan-1 is localized on villi of hepatocytes located in the space of disse. All four types of syndecan-encoding genes are expressed in hepatocytes, but only syndecan-1 can bind to lipoproteins. It was suggested that syndecan-1 in hepatocytes possesses a significant amount of sulfated sugars and, correspondingly, greater negative charge in comparison with other syndecans. [56]

V. Statins:

V.1. Types:

Statins are potent inhibitors of cholesterol biosynthesis used extensively to treat patients with hypercholesterolemia. Currently available statins may be classified into two groups, natural and synthetic statins. Natural statins include lovastatin, which is a fungal metabolite, and its synthetic derivatives, pravastatin and simvastatin. Fluvastatin, atorvastatin and rosuvastatin are fully synthetic compounds with completely different chemical structure. [57]

V.1.1 Simvastatin:

V.1.1.1. Chemistry of Simvastatin:

Simvastatin is butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8 α-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2 H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1 S - [1α,3α,7β,8β(2 S*,4 S*),-8 αβ]]. Simvastatin is a white to off-white, non-hygroscopic, crystalline powder that is practically insoluble in water, and freely soluble in chloroform, methanol and ethanol. The empirical formula of simvastatin is $C_{25}H_{38}O_5$ and its molecular weight is 418.57 g/mol. [58] its structural formula is illustrated in **figure (5):**

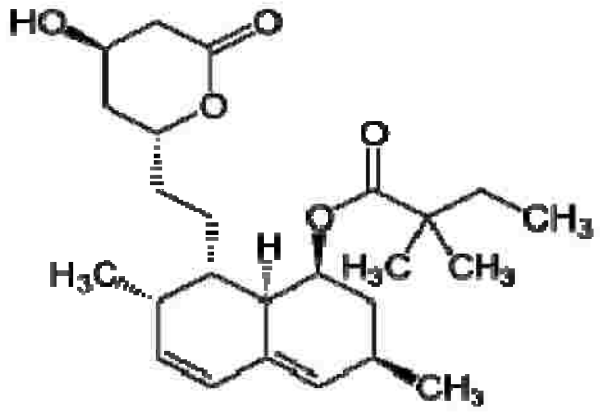

Figure (5): Structural formula of simvastatin. [58]

V.1.1.2. Mechanism of Action:

Simvastatin is a prodrug and is hydrolyzed to its active β-hydroxyacid form, simvastatin acid, after administration. Simvastatin is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the biosynthetic pathway for cholesterol. In addition, simvastatin reduces VLDL and TG and increases HDL-C. [59]

V.1.1.3. Pharmacokinetics:

Simvastatin is a lactone prodrug, upon incubation with liver microsomes four major metabolic products were formed (3'-hydroxy, 6'-exomethylene, 3', 5'-dihydrodiol and the active hydroxy acid of simvastatin), together with several minor unidentified metabolites. The 3', 5'-dihydrodiol simvastatin a new metabolite, was inactive as an inhibitor of HMG-CoA reductase. [60]

Kinetic studies of simvastatin metabolism in human liver microsomes suggested that the major NADPH-dependent metabolites (3'-hydroxy, 6'-exomethylene, and 3', 5'-dihydrodiol simvastatin) were formed with relatively high intrinsic clearances, consistent with the extensive metabolism of simvastatin observed in vivo. Based on four different in vitro approaches, it is concluded that CYP3A is the major enzyme subfamily responsible for the metabolism of simvastatin by human liver microsomes. [61]

Following an oral dose of simvastatin, 13% of the dose was excreted in urine and 60% in feces. Plasma concentrations of total simvastatin (simvastatin plus metabolites) peaked at 4 hours and declined rapidly to about 10% of peak by 12 hours postdose. [62]

V.2. Effect of statins:

Cholesterol biosynthesis is a complex process that involves more than 30 enzymes with five major stages. This biosynthetic route commences with the condensation of acetyl-CoA and acetoacetyl-CoA to form HMG-CoA. Subsequently, HMG-CoA is reduced to mevalonate (mevalonic acid) in a NADPH-dependent process by the enzyme HMG-CoA reductase. Mevalonate is phosphorylated to isopentenyl pyrophosphate and to other active isoprenoids (geranyl and farnesyl pyrophosphates), which condense and combine to

form squalene. Squalene is converted to lanosterol, which, after a number of reactions, is finally converted to cholesterol. Several important enzymes regulate this pathway; however, HMG-CoA reductase appears to be the rate-limiting enzyme of the entire process and so for endogenous cholesterol biosynthesis. (63)

Statins are competitive inhibitors of HMG-CoA reductase. Thus, statins produce a reduction in the formation of mevalonate, resulting in decreased hepatic cholesterol synthesis. By inhibiting HMG-CoA reductase, statins reduce the hepatocyte cholesterol content, stimulate expression of LDL receptors and ultimately enhance the removal of circulating LDLs associated to cholesterol. Abnormally elevated levels of circulating cholesterol and LDL-C are widely accepted to significantly contribute to the formation of atherosclerotic plaques and are involved in associated diseases. Inhibition of mevalonate synthesis also causes a decrease in the formation of other intermediates in the cholesterol synthetic pathway, such as geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP), with a subsequent reduction in geranylgeranyl pyrophosphate (GGPP). These isoprenoid-like molecules are involved in post-translational modification of proteins, termed isoprenylation, serving as important lipid-attachment molecules for post-translational modification of several proteins including heterotrimeric G and small GTP-binding proteins such as Ras, Rac, and Rho. Geranylygeranylation is of particular interest as it affects G-protein–dependent cellular activation and numerous signaling pathways involving Rac and Rho proteins. Reduction of protein isoprenylation is the molecular mechanism underlying most lipid lowering-independent effects of statins, collectively termed pleiotropic effects. Cholesterol biosynthesis and cell mevalonic acid pathway and modulation of various signaling pathways by statins are shown in (**figure 6**). (64)

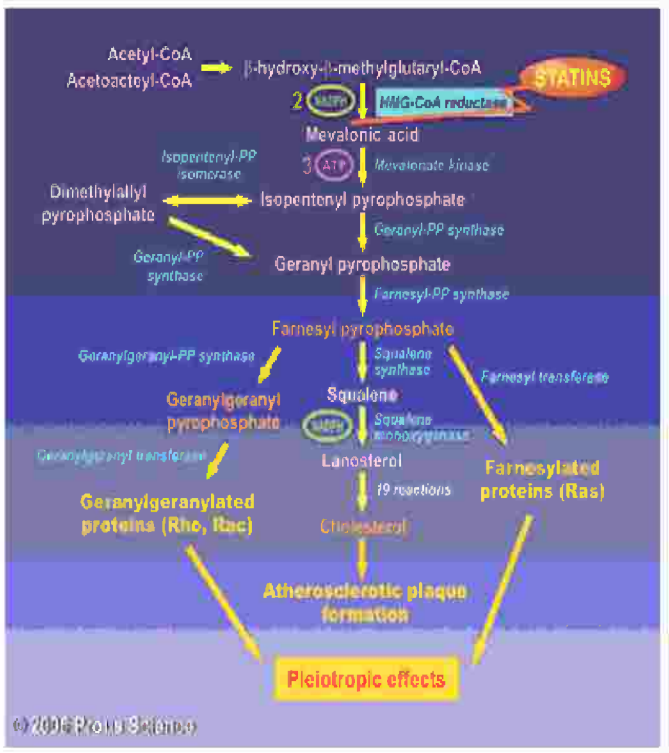

Figure (6): Diagram of the cholesterol biosynthesis pathway. By preventing isoprenylation of small GTPase proteins, HMG-CoA reductase inhibitors lead to modulation of various signaling pathways. (64)

V.2.1. Pleiotropic effect of statins:

Several trials have demonstrated beneficial effects of statins in lowering cardiovascular-related morbidity and mortality in patients with coronary artery disease, while beneficial effects of statins are assumed to result from competitive inhibition of cholesterol synthesis, a growing body of evidence supports the beneficial effects of statins independent of their ability to lower serum cholesterol levels. [65]

Although lipid lowering is certainly an important beneficial effect of statins in ameliorating micro/macrovascular complications of diabetes, pleiotropic effects of statins appear to be just as important. [64]

The mechanisms underlying the pleiotropic effects of statins are shown in **figure (6)**. The pleiotropic effects of these drugs include direct beneficial effects of statins on endothelial function, stabilizing atherosclerosis plaques, ameliorating progression of nephropathy and bone disease, and improvement in insulin sensitivity and the development of diabetes. By inhibiting HMG-CoA reductase, statins exert various other effects that include suppression of cytokine expression, induction of apoptosis, inhibition of cell proliferation, interference in the intracellular signaling, and modulation of extracellular matrix (ECM) protein degradation, all of which may play important roles in the pathogenesis of micro/macro complications of diabetes .[65, 66]

V.3. Statins and endothelial dysfunction in diabetes:

Growing evidence indicated that Akt, a serine-threonine kinase protein and a potential target of hyperglycemia, may be an important cytokine involved in pathogenesis of diabetic microangiopathy. Akt has been shown to activate endothelial nitric oxide synthase (eNOS) through increasing the affinity of eNOS to calmodulin. As Akt is activated by insulin binding to endothelial cells, hyperglycemia and insulin resistance may down-regulate the activity of Akt/PKB pathway leading to decreased NO activation. [67]

Although it was initially thought that the beneficial effects of statins on endothelial dysfunction were related to their lipid lowering properties, many studies indicated that the protective effects of statins on endothelial function may be mainly by increasing nitric oxide biosynthesis and bioavailability via the direct effect of statins to up-regulate the expression of eNOS. [67]

The Akt/PKB pathway may prove to be crucial for the observed beneficial effects of statins on diabetic induced endothelial dysfunction. A recent study suggested that the beneficial effects of statin on endothelial dysfunction may be the result of statin-induced activation of protein kinase Akt. Statins reverse the inhibitory effect of hyperglycemia on Akt/PKB pathway through phosphorylation and activation of Akt, thereby restoring eNOS activity and ameliorating endothelial dysfunction in diabetic milieu. [68] Statins and Akt/PKB pathway is shown in **(figure 7).**

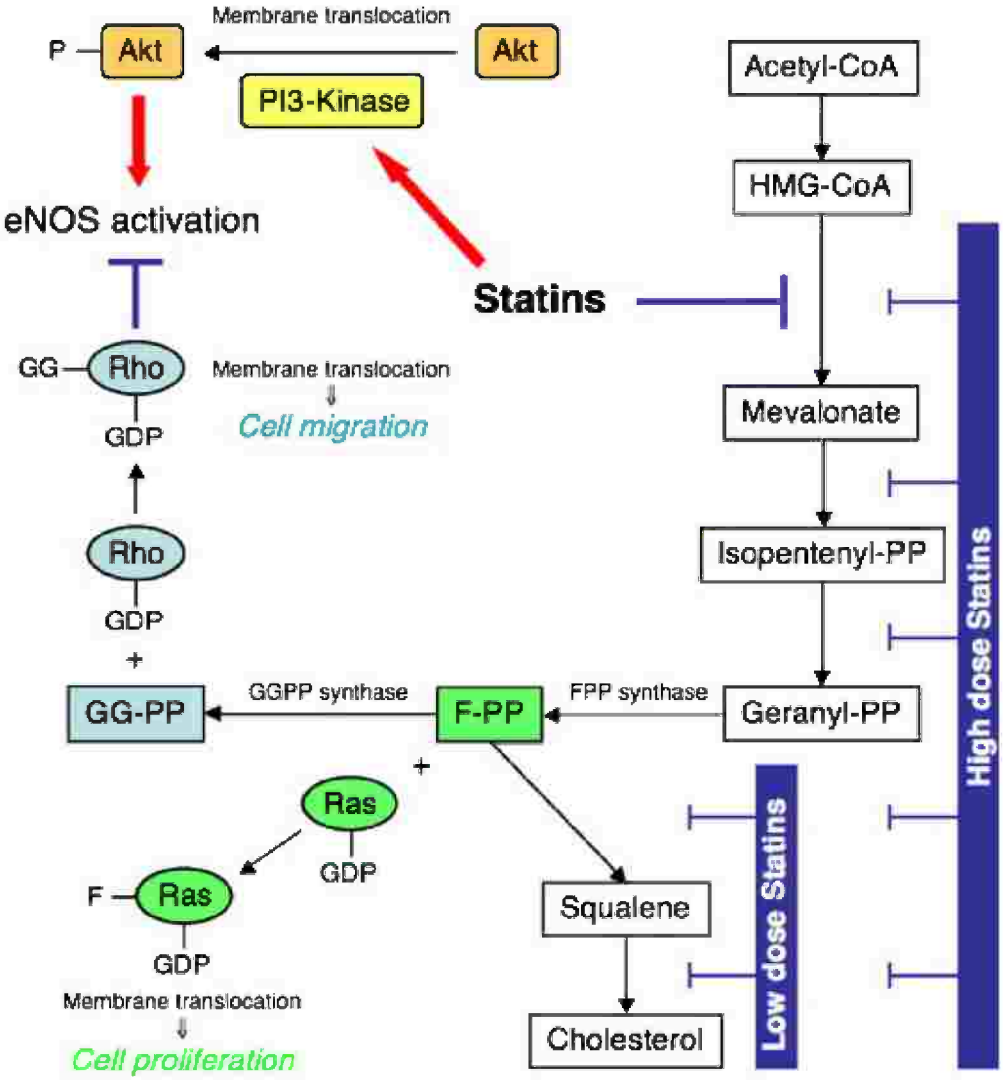

Figure (7): Cholesterol synthesis cascade with non-sterol products, FPP and GGPP, and working sites of statins. *FPP, farnesyl pyrophosphate; GGPP, geranyl geranylated pyrophosphate; eNOS, endothelial nitric oxide synthase.* [68]

V.4. Statins and diabetic induced oxidative stress:

The protective effect of statins on reactive oxygen species-(ROS) induced angiopathy is multifactorial and may include non cholesterol- dependent as well as cholesterol-dependent anti-oxidative properties. It has been shown that statins down-regulate macrophage scavenger receptors, thus reducing oxidized LDL-C uptake and the formation of foam cells within the intima. Statins also attenuate endothelial ROS formation through attenuating endothelial superoxide anion formation by inhibiting NADH oxidases via Rho-dependent mechanisms. Some of the anti-oxidative effects of statins may be due to metabolites of statins such as the hydroxyl metabolites of atorvastatin, which has been shown to have potent antioxidant properties. Statins have also been shown to improve and preserve the levels of important antioxidants such as vitamin C and E. [69]

V.5. Statins and diabetic nephropathy:

Diabetic nephropathy (DN) is a common complication of type 1 and type 2 diabetes, and it remains the single most common cause of renal failure. The onset of proteinuria in diabetic patients is not only an indicator of renal morbidity, but is also associated with a dramatically increased risk of premature cardiovascular disease and increased mortality.[34]

It has recently been suggested that statins may confer renoprotection in a variety of glomerular diseases including DN through their lipid-lowering properties. The beneficial effects of statins on renal function has been demonstrated in animal models of diabetes, including the obese Zucker rats and db/db mice, established animal models of type 2 diabetes. Human studies, however, had success in demonstrating attenuated progression of DN with statins. In support of a beneficial effect of statins in ameliorating the progression of DN, there are many reported findings of a preserved glomerular filtration rate (GFR) and serum creatinine in diabetic patients with proteinuria treated with lovastatin. Other studies of diabetics with chronic renal insufficiency and hyperlipidemia also found decreased progression of DN in patients allocated to statin therapy. [70]

V.6. Statins and inflammation in diabetes:

Levels of C-reactive protein are elevated in diabetic patients, and have been shown to correlate with markers of endothelial dysfunction. Restoring normal endothelial function in diabetic patients may have important beneficial effects in reducing cardiovascular risk this is because the fact that CRP appears to be correlated to heart disease risk, Inflammation of the arteries, heart attack, stroke, and peripheral arterial disease. HMG-CoA reductase inhibitors may prove to be key inhibitors of low-grade inflammation and endothelial dysfunction by reducing inflammatory cell signaling. Recently it has been reported that atorvastatin improves endothelial-dependent vasodilatation in diabetic patients, and this improvement correlated with significant decrease in CRP levels. [71]

V.7. Effect of statins on syndecan-1:

Till now, very little is known about the relation between statins and syndecan-1 expression or shedding. Exclusively, a previous orphan study of Janosi et al., [72] indicated that mevastatin-induced the shedding of syndecan-1 from the surface of myeloma cells. Syndecan-1 mediates specific adhesion of myeloma cells to type I collagen, moreover, inhibits the in vitro invasion of malignant plasma cells into type I collagen and mediates cell-cell adhesion between myeloma cells. Mevastatin-induced syndecan-1 shedding from the surface of myeloma cells in culture and inhibited myeloma cell growth and induced apoptosis in vitro. This finding had been confided the anti-tumor effect of statins via the syndecan-1 shedding which can be used as an independent prognostic factor for the multiple myeloma. [72]

Accordingly, as inflammation and increased risk for cardiovascular disease are characteristic features of type 2 diabetes mellitus, there is a rationable for targeting prevention of pathologic inflammatory events associated with type 2 diabetes using statins through acceleration of syndecan-1 shedding.