1. Introduction and Aim

1.2.1 Type 1 diabetes mellitus

Formerly known as insulin-dependent diabetes mellitus (IDDM), childhood diabetes, this type of diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of cases of diabetes affecting children and young adults and accounts for approximately 15% of the total diabetic population. About 40% of people with type 1 develop severe nephropathy and kidney failure by the age of 50. It is characterized by the development of ketoacidosis in the absence of insulin therapy or insulin deficiency caused by autoimmune or idiopathic destruction of pancreatic β-cells, abrupt onset of symptoms and absolute dependence on exogenous insulin not only to control the hyperglycemia but to prevent the occurrence of ketoacidosis and sustain life.

Type 1 diabetes is thought to be inherited in genetically susceptible individuals by environmental factors such as viral, toxic or chemical agents that lead to autoimmune destruction of b-cells, resulting in the formation of altered protein components. This material is a foreign antigen to the immuno system, establishing the basis for an autoimmuno reaction against the cell of origin the b-cell.

1.2.2 Type 2 diabetes mellitus

More than 90% of all diabetes with unknown specific etiology, but hereditary factors, aging, and obesity are important risk factors for the previously known as non-insulin dependent diabetes mellitus (NIDDM), adult-onset diabetes mellitus, maturity-onset diabetes mellitus. Two groups of patients with NIDDM were recognized by different body composition, obese and no obese. Apart from these two gruous, a third group called maturity-onset diabetes of the young (MODY) had been described for those individuals in whom the diagnosis of diabetes is made before the age of 25 years. With the disease being non-ketosis rarely leads to ketoacidosis and typically response to diet and/or sulfonylurea urea drugs.

Family history for Type 2 diabetes is important factor and begins in middle life or beyond, often over the age of 40. Symptoms being more gradually than in IDDM, and the diagnosis is frequently discovered when an asymptomatic person is found to have elevated plasma glucose on routine laboratory examination. In contrast to insulin-
1. Introduction and Aim

dependent disease, plasma insulin levels are normal to high although there are an inability of insulin to lower plasma glucose levels effectively an-abnormality termed insulin resistance. Type 2 diabetes can result from genetics defects that cause both insulin deficiency and insulin resistance (a term refers to impaired tissue response to insulin) occurs during the early phase of NIDDM, but the disease frequently goes undiagnosed for many years because hyperglycemia during the earlier stages is not severe enough to cause symptoms.\textsuperscript{7,11}

The pathophysiological changes in type 2 diabetes are due to abnormal insulin secretion and resistance to insulin action in target tissues. Although either defects may be the initial pathogenic event that ultimately leads to the disease, most patients with the fully developed syndrome show impairments of both insulin secretion and insulin-mediated glucose disposal (insulin or insulin antibodies) receptors (decreased number or diminished binding of insulin) or post receptor (Abnormal signal transduction especially failure to activate tyrosine kinase) abnormalities. Most common cause of insulin resistance is obesity.\textsuperscript{12,13} The comparison between type 1 and type 2 is showed in table 1.
Table 1: Classification of Diabetes Mellitus

<table>
<thead>
<tr>
<th>I. Type 1 diabetes* (b-cell destruction, usually leading to absolute insulin deficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Immune mediated</td>
</tr>
<tr>
<td>2. Idiopathic</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Type 2 diabetes* (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>III. Other specific types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Genetic defects of b-cell function</td>
</tr>
<tr>
<td>2. Genetic defects in insulin action</td>
</tr>
<tr>
<td>3. Diseases of the exocrine pancreas</td>
</tr>
<tr>
<td>4. Endocrinopathies:</td>
</tr>
<tr>
<td>• Acromegaly</td>
</tr>
<tr>
<td>• Cushing's syndrome</td>
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<tr>
<td>• Glucagonoma</td>
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<tr>
<td>• Hyperthyroidism</td>
</tr>
<tr>
<td>• Somatostatinoma</td>
</tr>
<tr>
<td>• Aldosteronoma</td>
</tr>
<tr>
<td>5. Drug- or chemical-induced</td>
</tr>
<tr>
<td>6. Infections</td>
</tr>
<tr>
<td>• Congenital rubella</td>
</tr>
<tr>
<td>• Cytomegalovirus</td>
</tr>
<tr>
<td>• Others</td>
</tr>
<tr>
<td>7. Uncommon forms of immune-mediated diabetes</td>
</tr>
<tr>
<td>• &quot;Stiff-man&quot; syndrome</td>
</tr>
<tr>
<td>• Anti-insulin receptor antibodies</td>
</tr>
<tr>
<td>• Others</td>
</tr>
<tr>
<td>8. Other genetic syndromes sometimes associated with diabetes</td>
</tr>
<tr>
<td>• Down's syndrome</td>
</tr>
</tbody>
</table>
1.3 The complications of diabetes mellitus

The complications are seen very less in people who have well-controlled blood sugar levels. In fact, the better the control, the lower the risk of complications. Hence patient education, understanding and participation are vital. Healthcare professionals who treat diabetes also address other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels (control with diet, exercise or medication), obesity (even modest weight loss can be beneficial), high blood pressure, and lack of regular exercise.

1.3.1 Acute complication of diabetes mellitus

Hyperglycemia is increased urination (polyuria), fatigue or blurry vision one of the main acute complication of diabetes. If uncorrected, hyperglycemia eventually may lead to diabetic ketoacidosis (DKA) or non-ketotic hyperosmolar coma. In actually, they represent parts of a spectrum of a disease process characterized by varying degrees of insulin deficiency over production of counter regulatory hormones and dehydration hypoglycemia, another acute complication of diabetes, results from an imbalance between the medication for diabetes treatment (insulin or sulfonylurea) and the patient's food intake or exercise. Because the brain depends almost entirely on glucose can lead to confusion, stupor or coma.

1.3.1.1 Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is an acute, dangerous complication and is always required medical emergency. On presentation at hospital, the patient in DKA is typically dehydrated and breathing both fast and deeply with abdominal pain. The level of consciousness is normal until late in the process, when lethargy (dulled or reduced level of alertness or consciousness) may progress to coma. The ketoacidosis can become severe enough to cause hypotension and shock. Prompt proper treatment usually results
1. Introduction and Aim

in full recovery, though death can result from inadequate treatment, delayed treatment or from a variety of complications. It is much more common in type 1 diabetes than type 2, but can still occur in patients with type 2 diabetes.\(^{17}\)

1.3.1.2 Non ketotic hyperosmolar coma

Hyperosmolar non-ketotic state (HNS) is another acute problem associated with diabetes mellitus. It has many symptoms in universal with DKA, but a different source, and requires different treatment. In very high blood glucose levels (usually considered to be above 300 mg/dl or 16 mmol/l), water will be cosmetically driven out of cells into the blood. The kidneys will also be "throwing away" glucose into the urine, resulting in simultaneous loss of water, causing an increase in blood osmolality. If the fluid is not replaced, the osmotic effect of high glucose levels combined with the loss of water will ultimately result in such a high serum osmolality (dehydration). The body's cells may become progressively dehydrated as water is drawn out from them and excreted. In addition, electrolyte alteration is also common. This combination of changes, especially if prolonged, will result in symptoms of lethargy (dulled or reduced level of attentiveness or consciousness) and can progress to coma. Urgent medical treatment is required in DKA, especially volume replacement. This is the diabetic coma which more usually occurs in type 2 diabetics. Coma in diabetes is due to acidosis and dehydration. Moreover, the blood glucose can be elevated to such a level that independent of plasma pH, the hyperosmolarity of the plasma is responsible for unconsciousness Accumulation of lactic acid in the blood (lactic acidosis) might be responsible for diabetic ketoacidosis if the tissues become hypoxic and lactic acidosis may itself cause coma.\(^{3}\)

1.3.1.3 Hypoglycemia

Hypoglycemia is a complication of several diabetes treatments. It can develop if the glucose intake does not match the treatment. The patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings similar to dread and immobilized panic. Consciousness can be changed, or even lost, in severe cases, leading to coma and/or convulsions or even brain damage and death. In patients with diabetes this can be caused by several factors,
such as too much or incorrectly timed insulin, too much exercise or incorrectly timed exercise (which decreases insulin requirements) or not sufficient food or inadequate amount of carbohydrates in food. Generally, hypoglycemia is treated with sweet food or drinks. In severe cases, an injection of glucagon (a hormone with the opposite effects of insulin) or an intravenous infusion of glucose is used for treatment, but usually when the person is unconscious.¹

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**Fig. 1: Acute effects of insulin deficiency (Diabetes Mellitus)**
1.3.2 Chronic complications of diabetes mellitus

The mostly diabetic patients are prone to an extensive array of medical complications; the majority of the problems can be attributed to particular susceptibility to damage to the kidney (nephropathy), the eye (retinopathy), the peripheral nerves (neuropathy), and the blood vessels (atherosclerosis). The first three categories of complication are relatively specific for diabetes and are characterized by pathologic endothelial changes, such as basement membrane thickening and increased vascular permeability. Therefore, nephropathy, retinopathy and neuropathy have been categorized as micro vascular complications of diabetes. The increased susceptibility to atherosclerosis and its complications are categorized as macro vascular complications.\textsuperscript{15}

The functional and structural changes in the involved organs usually lead in turn to the development of well-defined clinical entities, the so-called "complications of diabetes", which affect the eye, the kidney and the nervous system\textsuperscript{18} suggest that, the term "complication" may be rejected, arguing that the tissue changes are an integral part of a "syndrome", preceding or even initiating hyperglycemia. Moreover, the diabetic complications can be classified into vascular complications (arteriosclerosis, eyes and kidneys), infections (there is lowering resistance towards infection especially if the diabetes is poorly-controlled)\textsuperscript{19} and coma (Ketoacidosis and non-ketoacidosis).

The disease occurs when insulin activity is deficient. Since the introduction of insulin for diabetic therapy and the subsequent increased longevity of diabetics, the vascular complications of diabetes playing a major role in morbidity.\textsuperscript{20,21} Vascular complications can be divided into two types; affecting glomeruli and arteriosclerosis involving the cranial arteries, coronary arteries and peripheral arteries.\textsuperscript{22} Involvements of the peripheral arteries frequently lead to claudication, amputation and ischemic ulcers.\textsuperscript{23} In fact, arteriosclerosis spears at an earlier age in diabetics, is more extensive and is associated with a higher morbidity and mortality.\textsuperscript{21,24,25}

1.3.2.1 Microvascular disease

Chronic elevation of blood glucose level responsible for damage of blood vessels. In diabetes, the resultant problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the
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The damage to small blood vessels leads to a microangiopathy, which causes the following organ-related problems:

Diabetic retinopathy, growth of friable and poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can be responsible for severe vision loss or blindness. Retinal damage makes it the most common cause of blindness among non-elderly adults in the US.

Diabetic neuropathy, abnormal and decreased sensation, usually in a stocking distribution starting at the feet but potentially in other nerves. When combined with damaged blood vessels this can lead to diabetic foot. Other forms of diabetic neuropathy may present as mononeuritis or autonomic neuropathy.

Diabetic nephropathy (DN) or nephropatia deabetica or Kimmelstiel-Wilson syndrome or nodular diabetic glomerulosclerois, is one of the most frequent life threatening complications of diabetes mellitus. It is the leading cause of chronic renal failure (CRF) and end-stage renal disease worldwide, eventually requiring dialysis. Diabetic nephropathy develops in close to 40% of patients with type 1 diabetes and in 5% to 40% of patients with type 2 diabetes. Various metabolic and hemodynamic factors interactions are mainly responsible for development of diabetic nephropathy which activates common pathway for kidney damage. Genetics play an important role: Patients who have one or two deletions of the angiotensin-converting enzyme (ACE) gene, a defect in the sodium proton pump, or a family history of hypertension are at increased risk for progression to diabetic nephropathy. However, in such patients, nephropathy does not occur until type 1 diabetes develops; the worse and more prolonged the hyperglycemia, the greater the risk of diabetic nephropathy.

1.3.2.2 Macrovascular disease

Macrovascular disease leads to cardiovascular disease, mainly by accelerating atherosclerosis:

✓ Coronary artery disease, leading to myocardial infarction ("heart attack")
✓ Peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot.
✓ Diabetic myonecrosis
Diabetic foot is mainly occur due to combination of neuropathy and arterial disease, may cause skin ulcer and infection and, in serious cases, necrosis and gangrene. It is the most common cause of adult amputation, usually of toes and or feet, in the US and other Western countries. However, diabetes does cause higher morbidity, mortality and operative risks with this conditions.27
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1.4 Oxidative stress

At the beginning of life, the organisms obtained their energy (ATP) by anoxygenic photosynthesis, for which oxygen was toxic. Most of the metabolic pathways were developed during this anaerobic stage of life, in which oxygen came later. Cyanobacteria started producing oxygen from photosynthesis, which raised the atmospheric oxygen, and favored those organisms which have developed into eukaryotic cells with mitochondria, capable to use oxygen for a more efficient energy production.\(^{28}\)

Whenever a cell’s internal environment is disturbed by infections, disease, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption. This “oxidative shielding” acts as a defense mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighboring cells.\(^{28}\) Therefore, ROS formation is a physiological response to stress.

The term “oxidative stress” has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal. Being highly reactive molecules, the pathological consequence of ROS and RNS excess is damage to proteins, lipids and DNA.\(^ {29}\) Consistent with the main role of ROS and RNS formation, this oxidative stress might be responsible for physiological dysfunction, cell death, cancer, pathologies such as diabetes, and aging of the organism.\(^ {30}\)

1.5 Free radicals and reactive oxygen/nitrogen species

The presence of an unpaired electron that makes these molecules highly unstable is known as free radicals. Unpaired electrons have a tendency to pair formation, thus free radicals are chemically highly reactive and have a short half-lifetime. As a consequence of their low concentrations and their short lifetime, they cannot be easily detected. Some of the free radical- derived or -related substances (e.g. hydrogen peroxide, singlet oxygen) do not have any unpaired electrons, despite they are highly reactive. Thus, these materials are no actual free radicals; they are called reactive oxygen/nitrogen species (ROS/RNS).\(^ {31}\)
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Detection of free radicals may require direct or indirect methods. The direct detection involved electron spin resonance spectroscopy (ESR) and the use of spin traps. These latter molecules trap the free radicals, and they form an adduct with a much longer lifetime. Triggering and relaxation of these free-radical adducts can be measured by ESR providing information on the amount and structure of the radicals.$^{32,33}$

An indirect method is to detect the free-radical-induced damage to molecules rather than the presence of the free radicals themselves. Such methods do not require an ESR device, but are less precise than the ESR measurements. Indirect methods for free-radical detection contain the measurement of utilization of antioxidants (e.g. Total Antioxidant Capacity, TAC or Total Antioxidant Status, TAS), that of thiobarbituric acid-reactive substances, lipid peroxidation products (e.g. isoprostanes) or amino acid oxidation products etc.$^{34,35}$

1.6 Free radical-reactions

1.6.1 Formation of the reactive oxygen species, reduction of molecular oxygen

Molecular oxygen has an electron pair on its outer orbital, therefore it is stable. The yield of free radicals requires activation of the oxygen molecule either by excitation (singlet oxygen), reduction (superoxide free radical, hydroxyl free radical, hydrogen peroxide), molecular scission (oxygen atom), or oxidation (molecular oxygen ion). The most common way is the partial reduction of oxygen.

The oxygen molecule can be reduced in a four-electron reaction to chemically inert water e.g. in the mitochondria during terminal oxidation by cytochrome enzymes. However, by a single-electron reduction of oxygen, superoxide free radical (・O$_2$ -) is formed. Such a partial reduction can take place when oxygen-containing solutions are excited by ionizing radiation or ultrasound. In addition, physiologically more appropriate mechanisms involve “autoxidation” of reducing sugars, non-enzymatic chemical reactions, and enzymatic reactions such as the NADPH-oxidase and the xanthine-oxidase reactions.

The ・O$_2$ - has a short lifespan, it can be converted into dismutated i.e. further reduced to form hydrogen peroxide (H$_2$O$_2$). The reaction may happen spontaneously, but it may be catalyzed by the superoxide dismutase enzyme (SOD). Although H$_2$O$_2$ is not a
1. Introduction and Aim

free radical, it is still reactive; therefore it belongs to the ROS, and it may give rise to the making of more reactive substances, as the hydroxyl free radical (·OH). The SOD reaction leads to a formation of a ROS from a free radical, i.e. a chemically less active substance is formed, therefore both isoforms of SOD, the cytosolic Cu/Zn-SOD and the mitochondrial Mn-SOD are regarded as antioxidant enzymes.

·OH is the most reactive of all free radical species, thus it has the shortest life-span. It is produced by the cleavage reactions of H₂O₂, such as the Haber-Weiss and Fenton reactions. ·OH may directly damage biologically important macromolecules, while itself becomes further reduced to water in these reactions.

As reactions of H₂O₂ can lead to ·OH formation it is important that there exist some routes by which H₂O₂ may be degraded before yielding ·OH. These reactions are catalyzed by the catalase enzyme and the peroxidases such as glutathione peroxidase.¹³,³⁶

1.6.2 The Haber-Weiss reaction:

In this reaction, H₂O₂ will be split to the more active free radical species, ·OH and to OH⁻.³⁶

\[
·O_2^- + H_2O_2 \rightarrow O_2 + ·OH + OH^- 
\]

1.6.3 The Fenton and Fenton-like reactions

The classical Fenton-reaction has been described as a cleavage reaction of H₂O₂ catalyzed by the redox cycling of ferrous iron. The simplified equation is:

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + ·OH + OH^- 
\]

Ferric iron and iron complexes are generally used in in-vitro experiments to model free radical production in Fenton-like reactions. To avoid Fenton-like reactions, iron is usually stored in a form of iron-protein complexes, such as transferrin or ferritin. The non protein-bound forms of iron may also be complexed by other substances as citrate, organic phosphates. These chelators may either enhance or suppress redox activity of iron.³⁶
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1.6.4 Damage of biologically important macromolecules as a consequence of free radical-attack

Free radicals are, as a consequence of their unique chemical structure highly reactive, they can attack biologically active molecules, such as lipids, carbohydrates, nucleic acids, proteins and amino acids.

Peroxidation of partially unsaturated fatty acids, membrane lipids may involve enzymatic and non-enzymatic pathways, as well. Oxidized metabolites of the arachidonic acid are formed mainly in enzymatic reactions, they are highly important messengers. The non-enzymatic reactions are fast, and unlike the enzymatic reactions, they are not that strictly controlled. Cyclic endoperoxides and lipid hydroperoxides are formed during the lipid peroxidation, which give rise to even smaller advanced lipid peroxidation end products (ALE) such as malondialdehyde, glyoxal, methylglyoxal, acrolein.\textsuperscript{35,37}

There is also a free-radical-related damage of nucleic acids and nucleotides mainly by $\cdot$OH which may lead to strand-breaks, hydroxylation of nucleotide bases (e.g. to 5-hydroxymethyl-uracil and 8-hydroxy-uracil), formation of thymidine-dimers and consequently mutations. These alterations may have a role in the pathogenesis of malignancies.\textsuperscript{38}

1.6.5 Damage to nitric oxide and formation of peroxynitrite

Nitric oxide (NO) is a potent vasodilator, an endothelium-dependent relax factor, which is also able to decrease platelet aggregation, leukocyte adhesion and vascular smooth muscle cell proliferation. Thus, NO is anti-thrombotic, protects against vascular remodeling and decreases blood pressure. NO is formed by the nitric oxide synthase (NOS) enzyme, that has three isoforms, the inducible NOS (iNOS) and the constitutively expressed endothelial (eNOS) and neural (nNOS) isoforms. The NOS enzyme cleaves the amino acid L-arginine (LArg) to NO and citrulline in a NADPH-dependent reaction. NO has a short lifespan, and it has been shown that it has an unpaired electron, i.e. it is one of the free radical species, as well. Decreased NO levels are described in kidney diseases, probably as a mediator and/or consequence of renal damage.\textsuperscript{39}

In the presence of $\cdot$O$_2$-, the two free radicals (NO and $\cdot$O$_2$-) may react with each other and form a substance that is called peroxynitrite (ONOO$^-$). The properties of
ONOO are the opposite of that of NO, namely ONOO is vasoconstrictor, pro-thrombotic and induces remodeling etc. In some pathological states NOS enzyme activity may be uncoupled from the L-Arg to NO conversion, in these states the redox reactions still take place, and the enzyme produces •O2-. This may further contribute to ONOO production.\textsuperscript{40}

1.7 Antioxidant defenses in the organism

As a small part the oxygen consumed for aerobic processes will be converted into superoxide anion\textsuperscript{41}, which will have to be scavenged or converted into less reactive (and harmful) molecules. The main enzymes that regulate this process are Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (Figure 3). When ROS overproduction or chronic hyperglycemia occurs, the activity of these enzymes is insufficient, leading to more ROS and RNS formation and activation oxidative stress pathways.

SOD is considered a first-line defense against ROS. This enzyme is present in nearly all cells, and converts •O2 - into H2O2. Mitochondrial and bacterial SOD contain Mn, while cytosolic SOD is a dimer containing Cu and Zn. As the H2O2 may still react with other ROS, it needs to be degraded by either one of the other two antioxidant enzymes, GSH-Px or catalase.\textsuperscript{42,43}
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GSH peroxidase is located in the mitochondria. It catalyzes degradation of H2O2 by reduction, where two glutathione (GSH) molecules are oxidized to glutathione disulfide (GSSG).

Regeneration of GSH by GSH-reductase, requires NADPH, which is oxidized to NADP+. Catalase, on the other hand, is localized primarily in peroxisomes, and so it detoxifies the H2O2 that diffuses from the mitochondria to the cytosol, converting it into water and molecular oxygen.\(^{42,43}\)

There are also nonenzymatic antioxidant mechanisms, which mostly help regenerate GSSG back into GSH. Antioxidant vitamins such as A, C, E and alpha-lipoic acid are among these mechanisms. Although all these antioxidant defenses work together to eliminate H2O2 (and thus superoxide) from the cell, in the presence of reduced transition metals (Cu, Fe), H2O2 can be transformed into •OH, which is a highly reactive ROS, by the Fenton reaction.\(^{42,44}\)

1.8 Coenzyme Q10

2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone i.e. Coenzyme Q10 or ubiquinone is a vitamin-like substance which is lipid soluble in nature and exists in the hydrophobic interior of the phospholipid bilayer of cell membrane. It exists in the wide
1. Introduction and Aim

range of dietary items including poultry, meat, fish, vegetable oils and nuts.\textsuperscript{45} The Coenzyme Q10 posses the chain of 9 or 10 isoprene units along with the quinone head.\textsuperscript{46} Delocalization of $\pi$-electrons, adjacent electron-donating heteroatoms and a long isoprenoid chain are the structural features of the Coenzyme Q10 (Figure 4) that help it to diffuse through the membrane phospholipid bilayer.\textsuperscript{47}

\begin{center}
\includegraphics[width=0.5\textwidth]{coenzyme_q10.png}
\end{center}

\textbf{Fig. 4: Structure of Coenzyme Q10}

Along with $\alpha$-tocopherol, it plays a vital role in scavenging of free radicals in mitochondrial electron transport chain to serve as in vivo antioxidant.\textsuperscript{48} During this process, it is reduced to semiquinone and ubiquinol, which has important function to generate superoxide anions during mitochondrial respiration.\textsuperscript{49} Coenzyme Q10 serves as an electron carrier between nicotinamide dinucleotide and succinate dehydrogenases and the cytochrome system.\textsuperscript{50} It has a potent anti-inflammatory property and acts through inhibition of release of pro-inflammatory cytokines i.e. tumor necrosis factor-$\alpha$.\textsuperscript{51} It has been earlier stated that coenzyme Q10 has protective effect in testicular injury medicated by sodium arsenite as well as magnetic field exposure.\textsuperscript{52,53} In addition, it has been also reported that coenzyme Q10 possess wide spectrum of activities including aging,\textsuperscript{54,55} antiulcer,\textsuperscript{56,57} in vivo antioxidant,\textsuperscript{58} antidiabetic,\textsuperscript{51} and antihypertensive activity.\textsuperscript{59,60}

1.9 Metformin

Metformin is an antidiabetic agent that decreases intestinal absorption of glucose, increases its anaerobic metabolism, improves insulin sensitivity and decreases glucagon release. In recent study, it was shown that metformin prevented oxidative stress-induced
1. Introduction and Aim

deat in several cell types, including endothelial cells through a mechanism dependent on the mitochondrial permeability transition pore opening.\textsuperscript{61,62} It was also reported that metformin attenuates Diabetic nephropathy by modulation of oxidative stress markers at the gene expression level, with the consequent improvement in mitochondrial function and inhibits the release of pro-inflammatory cytokines such as TNF-α and IL-6 genes.\textsuperscript{63} Moreover, metformin have an effect on the carbonyl stress which prevents AGEs formation and improves the free radical defense system.\textsuperscript{64} It was also found that metformin is responsible for to decrease the expression of growth factors such as TGF-β which plays an important role in the pathogenesis of DN.\textsuperscript{65}

1.10 Sitagliptin

Food and Drug Administration (U.S.) in October 2006 has approved sitagliptin as first dipeptidyl peptidase-IV (DPP-IV) inhibitor for the treatment of type 2 diabetes.\textsuperscript{66} After ingestion of meal it improves levels of incretin hormones like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino-tropic peptide (GI P).\textsuperscript{67} Both GLP-1 and GIP hormones increase glucose-dependent insulin biosynthesis and release of glucagon-like peptide-1, additionally inhibit glucagon secretion, delays gastric emptying.\textsuperscript{68} DDP-IV inhibitors have shown to enhance glycemic control, insulin secretion and proliferation and differentiation of pancreatic beta cells in animal models.\textsuperscript{69} These effects may be advantageous in preserving the pancreatic beta cell mass and function.\textsuperscript{70} DPP-IV inhibitors are helpful in acute renal failure and chronic renal disease.\textsuperscript{71} Co-administration of DPP-IV inhibitor (linagliptin) with telmisartan caused marked reduction in albumin urea, an early marker for DN and also decrease in tumor necrosis factor alpha (TNF-α) level, which is found during hyper filtration stage in diabetic nephropathy.\textsuperscript{72} It was previously shown that sitagliptin ameliorates renal ischemia reperfusion induced renal damage in diabetes.\textsuperscript{73} Long term use of sitagliptin treatment ameliorated all lesions glomerular, tubule-interstitial, and vascular of kidney.\textsuperscript{74}

1.11 Rosuvastatin

The 3-hydroxy-3-methylgluraryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have pleiotropic effects on cerebrovascular, cardiovascular, and micro-vascular
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Diseases independent of their cholesterol-lowering effect. It was earlier reported that statins have beneficial effects on kidney disease including diabetic nephropathy. Various studies have shown that statins prevented the progression of microalbuminurias and nephropathy in diabetes. Many of the beneficial effects occur independently of cholesterol-lowering effects because they are able to alter cellular proliferation/apoptosis, down-regulate inflammatory chemokine production, decrease reactive oxygen species generation, and reduce neutrophils and macrophage recruitment.

1.12 Aim of the study

Diabetic complication such as nephropathy, neuropathy and retinopathy due to type II diabetes may occur after a prolonged period of exposure of the high amount of glucose to the blood vessel and internal organs. This is because of glucose toxicity, otherwise called as glycosaled end products. Even the patients respond to all anti-diabetic drugs, there are few patients who are susceptible to diabetic complications as a very early stage. Several studies have reported that oxidative stress play major role in developing the diabetic complications. It was previously shown that diabetic dyslipidemia plays a pathogenetic role in the development and perpetuation of diabetic nephropathy (DN). Lipids may induce renal injury by stimulating transforming growth factor-β, thereby inducing the production of reactive oxygen species (ROS) and causing damage to the glomeruli and glomerular glycocalyx. Triglycerides-rich lipoproteins (TGRLs) can activate monocytes, degrade glycocalyx, and increase permeability of the glomerular filtration barrier, which may contribute to the progression of diabetic nephropathy. Therefore, there is a need of more and more anti-diabetic or antihyperlipidemic drugs in combination with antioxidants which can delay the onset of diabetic complication, thereby increasing the lifespan and quality of life of diabetic patients. Therefore, it was thought to combine antioxidant like coenzyme Q10 with anti-diabetic or antihyperlipidemic drugs to study their renoprotective and neuroprotective effect in experimentally induced nephropathy and neuropathy.