# CHAPTER I

# INTRODUCTION

**Background of the problems**

The kidneys are paired organs with several functions. They are an essential part of the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure. They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium; the kidneys also are responsible for the re-absorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, renin, and erythropoietin.

One cause of kidney failure is diabetes mellitus, a condition characterized by high blood glucose (sugar) levels. Over time, the high levels of sugar in the blood damage the millions of tiny filtering units within each kidney. This eventually leads to kidney failure [1]. Around 20 to 30 % of people with diabetes are at a higher risk of developing kidney disease (diabetic nephropathy) [2].

Diabetes mellitus is a common metabolic diseases characterized by high blood levels, that result from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glysuria, polyuria, and proteinuria. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses. Mechanisms which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycation end products (AGEs). Glycation protein can give an electron to the molecular oxygen, leading to oxygenated free radical [1, 3, 4].

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals [4]. This atom or molecules can form covalent bond with other atoms or molecule gathering. The example of free radical is $O_2^{-\bullet}$. When the $O_2$ oxidized, it can be cause the formation of reactive oxygen species both radical and non radical, particularly hydroxyl radical

($OH^{\bullet}$), superoxide anion radical ($O_2^{-\bullet}$) and hydrogen peroxide ($H_2O_2$). Reactive oxygen species are formed continuously as consequence of biochemical reactions as well as external factors which cause lipid peroxidation of cell and organelle membranes and, hence, disruption of the structural integrity and imbalance in cell capacity for cell transport and energy production, especially in the proximal tubule segment of kidney [3].

Major compounds of cell membranes are phospholipid, and their polyunsaturated fatty acid (PUFAs), because of their conjugated double bonds are the first target of free radical. One of the main forms of damage resulting from oxidative stress is lipidperoxidation [5]. Free radicals for example, hydroxyl, lipoxyl and lipid peroxyl are oxidized by unsaturated covalent bond from PUFAs to be PUFA radical ($L^{\bullet}$) and decomposition to conjugated diene for more stable or add with others radical to non-reactive complex. When lipid radical ($L^{\bullet}$) decomposition to conjugate diene then add with $O_2$ become lipid peroxide ($LOO^{\bullet}$) and damage unsaturated covalent bond of PUFAs and produce lipid hydroperoxide (LOOH) and final product of lipid peroxidation is malondialdehyde [4, 6]. Lipid peroxidation can perturb membrane function and contribute to loss of cellular functions for example, lost of flexibility, change function of enzyme and receptor and change ion channel and permeability of $Ca^{2+}$ [7].

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids which can be initiated a chain of events resulting in the onset of a variety of diseases. In living organisms, they have developed the antioxidant system for converting free radicals into non-toxic molecules. The cellular antioxidant can be divided into 2 major groups, enzymatic and non-enzymatic [8]. Enzymatic system including superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), and nonenzymatic including albumin, ceruloplasmin, ferritin, ascorbic acid, α-tocopherol, β-carotene, reduce glutathione, uric acid and bilirubin [9]. The cooperation among different antioxidant provides greater protection against cellular damage by reactive oxygen or nitrogen species. Thus, the overall of antioxidant capacity may provide more information compared to that obtained by the measurement of individual components [9].

Biochemical markers including serum creatinine, urea nitrogen and urine markers of kidney injury (casts, fractional excretion of Na) are insensitive and nonspecific for the diagnosis of acute tubular necrosis (ATN) and incremental increase in serum creatinine over a defined and relatively short time interval [10]. Unfortunately, changes in serum creatinine occur well after acute kidney injury has been sustained. The N-acetyl-β-D glucosaminidase (NAG activity) was measured as a urinary biomarkers for the early detection of acute kidney injury using for the diagnosis of ATN [11, 12].

**Problems**

Diabetes mellitus is a syndrome of metabolism disorder usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia), that result from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glucosuria, polyuria, and proteinuria. It is well-established fact that diabetes is a chronic disease and causes harmful complication. Insulin is a hormone produced in the pancreas, an organ near the stomach. Insulin is needed to turn sugar and other food into energy. Diabetes mellitus is caused by absolute or relative insulin deficiency, sometimes associated with insulin resistance, β-cell dysfunction, impaired glucose tolerance. Insulin resistance is an inability of some of the cells of the body to respond to insulin, especially by muscle and fat (adipose) tissues. At present, diabetes care is developed, the mortality rate is decrease but there are many complications in diabetic patients from the result of prolong high blood sugar levels in circulation [13, 14], that cause both acute and chronic complications [15]. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses. Mechanisms which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycation end products (AGEs). Protein glycation and advanced glycation end products (AGEs) results from the formation of a covalent binding between the aldehyde glucose function and the free amino groups of protein. Glycation protein can give an electron to the molecular oxygen, leading to oxygenated free radical [16].

Diabetes mellitus, serious long-term complications are microvascular and macrovascular complication that cause of damage of the other organs. The major long-term complications such as vision damage due to diabetic retinopathy, damage of nerve system or diabetic neuropathy cause loss of sensation or pain, renal failure due to diabetic nephropathy and loss of flexibility of vascular cause cardiovascular disease [15, 17]. These major factor that cause chronic complication of cardiovascular in those type 2 diabetic patients is oxidative stress from elevated of free radical.

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals [4]. The example of free radical is $O_2^-$, particularly hydroxyl radical ($OH^·$), superoxide anion radical ($O_2^{-·}$) and hydrogen peroxide ($H_2O_2$). When the $O_2$ oxidized, it can be cause the formation of reactive oxygen species (ROS) [4]. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids. Reactive oxygen species are formed continuously as consequence of biochemical reactions as well as external factors which cause lipid peroxidation of cell and organelle membranes and, hence, disruption of the structural integrity and imbalance in cell capacity for cell transport and energy production, especially in the proximal tubule segment of kidney [3].

Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. In recent years numerous clinical and experimental studies focused on detection of signs of oxidative stress in renal patients. There is good evidence indicating that ureamia in general is associated with enhanced oxidative stress. Renal sources for ROS are activated macrophages, vascular cells and various glomerular cells. This effect plays a role in a variety of renal diseases contribute to the pathogenesis of ischemia reperfusion injury in the kidney such as glomerulonephritis and tubulointerstitial nephritis, which can contribute to proteinuria and other conditions. This suggests that an increase in oxidative stress is considered an important pathogenic mechanism in the development of ischemic and toxic renal tubular injury [18, 19, 20]. ROS also impair enzymatic and structural protein molecules affect cellular function and vitality. For example, peroxidation of lipids in plasma and intracellular membranes perturbs membrane fluidity, permeability, and ion and solute transport, hydrogen peroxide compromises mitochondrial ATP synthesis by

inhibiting the ATP- synthetase complex. These changes are by the elevation in intracellular calcium, disruption of the cytoskeleton, foam cell formation of the plasma membrane, and finally cell death. Augmentation in renal production of ROS may be derived from metabolic and other processes engendered in endogenous cells in the injured kidney, or from activated leukocytes in the kidney with ATN That reactive oxygen species contribute to post ischemic renal injury [3].

In this research we determined oxidative stress parameter, has brought substantial insights into their pathogenesis. The methods available for evaluation of oxidative stress and antioxidant may divided into three categories (1) base on measuring the concentrations of peroxidation product such as lipid hydroperoxide, malondialdehyde and reactive oxygen species production, (2) based on evaluate the oxidative or reductive potency of a biological fluid and (3) based on the susceptibility of lipid peroxidation to various component of body fluids.

**Purpose of the Study**

In diabetes and impaired glucose tolerance, the proteins in body are glycated nonenzymatically at a higher rate in comparison to healthy subjects [21]. The glycated proteins undergo modification to produce advance glycation end products (AGEs) [22]. AGEs can bring about protein cross-linking and generation of free radical. Augmented AGEs are toxic to cell and are incriminated for diabetic complication. It has been recognized that oxidative stress is another factor hyperglycemia that enhance protein glycation and accumulation of AGEs in renal disease patients [23] are reported an aftermath of prevailing oxidative stress. The methods available for evaluation of oxidative stress and antioxidant may divided into three categories (i) base on measuring the concentrations of peroxidation product such as lipid hydroperoxide, malondialdehyde and reactive oxygen species production, (ii) based on evaluate the oxidative or reductive potency of a biological fluid and (iii) based on the susceptibility of lipid peroxidation to various component of body fluids. Phospholipid is major component of cell membrane and lipid peroxidation is the first target of the attack or free radical as describe above. Thus, the most used criteria of oxidative stress were determination of the concentration of lipid peroxidation product and the susceptibility of lipid peroxidation [24]. The goal of this research was to find the

evidence and baseline of oxidative stress and total antioxidant activity, and correlation with other variables of these patients. The measurements of MDA levels and total antioxidant activity in serum will be useful identified the association of those metabolic, oxidative stress biomarkers with the renal function markers (urinary NAG, creatinine, creatinine clearance levels and microalbumin).

The hypothesis of this research is (1) identify the prevalence of the renal disease and the metabolic and oxidative status markers (lipid hydroperoxide, malondialdehyde) in those participants. (2) compare those metabolic, oxidative status markers, and the renal function markers in those participants and (3) also identified the association of those metabolic, oxidative stress biomarkers with the renal function markers (urinary NAG, creatinine, microalbumin, estimated creatinine clearance; eCrCl levels and glomerular filtration rate; eGFR).