INTRODUCTION

Diabetes mellitus, a chronic metabolic disease, is characterized by an increase in the blood-glucose level resulting from a relative insulin deficiency or insulin resistance or both. As a consequence, it can lead to glycation of tissues, which proceeds with acute metabolic disturbances and ends with organ damage with severe health deteriorations. Research studies over the years, reported that the worldwide prevalence of diabetes mellitus appears to be increasing alarmingly. It is estimated that 5.4% of total population would be affected with the disease by the year 2025 as initial reports showed 4.0% in the year 1995. Thus, proper management should be done in order to treat diabetes mellitus and its complications ***(Peirce, 1999).***

Type 2 Diabetes may remain undiagnosed for many years because of the gradually developing hyperglycemia and at the time of diagnosis chronic diabetic complications can already be present. Classically, long-term complications of diabetes are divided into macrovascular (coronary artery disease, peripheral vascular disease and cerebrovascular disease) and microvascular complications (diabetic nephropathy, neuropathy and retinopathy) and represent, with direct and indirect effects of chronic hyperglycemia on vascular vessels, the major source of morbidity and mortality in T2DM. Therefore, prediction and prevention of chronic diabetic complications represent two major objectives necessary to improve the quality of life of type 2 diabetic patients ***(Sebastiani et al., 2013).***

Emerging evidence shows that mediators and effectors of insulin–IGF-1 signaling, including insulin receptor substrate 2 (IRS2), PI3K, 3-phosphoinositide-dependent protein kinase 1 (PDPK1), forkhead box protein O1 (FOXO1) and AKT kinases, have important roles in β-cell growth and function. Mammalian cells express four FOXO isoforms: FOXO1, FOXO3, FOXO4 and FOXO6, of which FOXO1 is the most abundant isoform in liver, adipose tissue and pancreatic β cells. FOXO1 is phosphorylated by AKT kinases, leading to its translocation from the nucleus to the cytoplasm, which in turn inactivates FOXO1 transcriptional activity. However, FOXO1 is also phosphorylated by other kinases, including mitogen-activated protein kinases (also known as JNKs), inhibitor of nuclear factor κB kinase (NFκB), and cyclin-dependent kinase ***(Kitamura, 2013).*** FOXO1 is a transcription factor that plays important roles in regulation of gluconeogenesis and glycogenolysis by insulin signaling, and is also central to the decision for a preadipocyte to commit to adipogenesis ***(Nakae et al., 2003).*** In its un-phosphorylated state, FOXO1 is localized to the nucleus, where it binds to the insulin response sequence located in the promoter for glucose 6-phosphatase and increases its rate of transcription. FOXO1, through increasing transcription of glucose-6-phosphatase, indirectly increases the rate of hepatic glucose production ***(Nakae et al., 2001).*** However, when FOXO1 is phosphorylated by Akt on Thr-24, Ser-256, and Ser-319, it is excluded from the nucleus, where it is then ubiquitinated and degraded. Phosphorylation of FOXO1 by Akt subsequently decreases the hepatic glucose production through a decrease in transcription of glucose 6-phosphatase.

Genomic analysis led to the identification of nephroblastoma overexpressed gene (Nov, also known as Ccn3) as a novel FOXO1 target ***(Paradis et al., 2013).*** The NOV/CCN3 (*Nephroblastoma Overexpressed*) gene was originally isolated as a target site for myeloblastosis associated virus in avian nephroblastoma, which is a unique animal model of the Wilm's tumour ***(Joliot et al., 1992).*** NOV/CCN3 is the third founding member of the CCN family of multitasking matricial proteins after CYR61/CCN1 (CYsteine Rich) and CTGF (Connective Tissue Growth Factor/CCN2). The human NOV/CCN3 protein is widely detected in the developing human embryo ***(Katsube et al., 2009).*** In adults, NOV is expressed in various tissues including the nervous system, adrenocortical glands, skeletal and cardiac muscle, cartilage, bone, lung and kidney. Within the vascular system, NOV expression has been demonstrated both in vascular smooth muscle cells and endothelial cells ***(Lin et al., 2010).*** The human NOV protein is a circulating molecule in amniotic fluid, cerebrospinal fluid and serum ***(Thaibout et al., 2003).***

Due to the critical role of FOXO1 factors in the regulation of gluconeogenesis and glycogenolysis, we hypothesized that the dysregulation in FOXO1 gene expression may be connected to the development of T2DM through working on its new target CCN3.

AIM OF THE WORK

1) Evaluate the expression of FOXO1 and its target CCN3 in patients with type 2 diabetes mellitus in a trial to explore the molecular mechanism underlying β cell failure in T2DM.
2) Correlate the relationship between the two gene expressions to each other and to the different clinicopathological factors and complications of T2DM.

DIABETES MELLITUS

A- Definition:

Diabetes mellitus (D.M) is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate metabolism resulting from defects in insulin secretion, insulin action or both. The effects of Diabetes Mellitus include long-term dysfunction and failure of various organs ***(Rother, 2007).***

B- Epidemiology and Prevalence:

In 2011, there were 366 million people with Diabetes Mellitus, and this is expected to jump to 552 million by 2030. Most people with diabetes live in low- and middle-income countries, and these countries will also see the greatest increase over the next 19 years ***(Whiting et al., 2011).***

An estimated 19.2 million people in the Eastern Mediterranean and Middle East Region (EMME), or 7% of adult population have diabetes. This is predicted to be more than double by 2025 and reach 39 million people, *figure (1)* ***(IDF, 2009).***

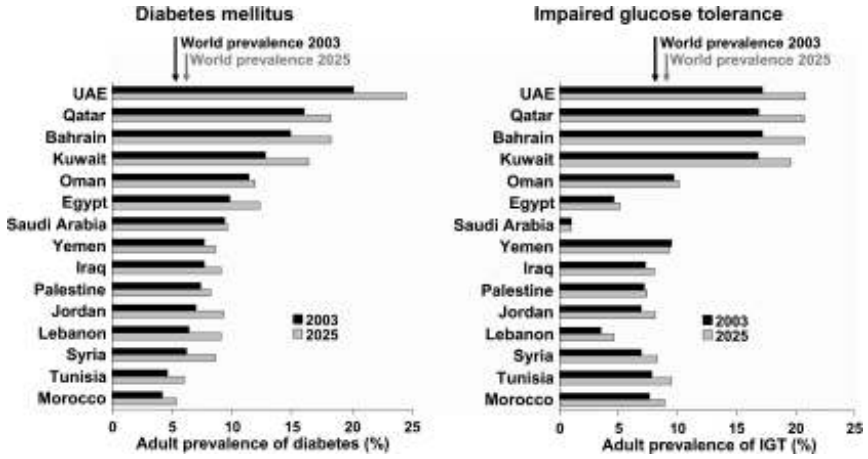

Figure (1): Increasing burden of dysglycaemia in the Middle East.

The International Diabetes Federation (IDF) has identified Egypt as the ninth leading country in the world for the number of patients with T2DM. The prevalence of T2DM in Egypt was almost tripled over the last 2 decades. This sharp rise could be due to either an increased pattern of the traditional risk factors for T2DM such as obesity and physical inactivity or other risk factors unique to Egypt as increased exposure to environmental risk factors like pesticides and increased prevalence of chronic hepatitis C ***(Hegazi et al., 2015)***.

C- Classification of Diabetes Mellitus:

In 1979 a work group of the National Diabetes Data Group proposed a classification scheme which recognized two major forms of diabetes: type I; insulin dependent diabetes mellitus (IDDM) and type II; non insulin dependent diabetes mellitus (NIDDM) ***(National Diabetes Data Group, 1979).*** This classification system went on to include evidence that DM

was an etiologically and clinically heterogonous group of disorders that share hyperglycemia in common. Such evidence was used by an International Expert Committee working under the sponsorship of the American Diabetes Association (ADA) to establish a classification based on the disease etiology rather than the type of pharmacological treatment. This classification includes type 1 DM, type 2 DM, specific types of diabetes, gestational DM (GDM), impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), *table (1)* ***(American Diabetes Association, 2007).***

Table (1): Etiological Classification of Diabetes Mellitus and other categories of glucose intolerance.

1- Type 1 diabetes
a. Immune mediated
b. Idiopathic
2- Type 2 diabetes
3- Other specific types of diabetes
a-Genetic defects of islet ß-cell function
b-Genetic defects of insulin action
c-Diseases of the exocrine pancreas
d-Endocrinopathies
e-Drug- or chemical- induced diabetes
f-Infections
g-Uncommon forms of diabetes
h-Other genetic syndromes
4- Gestational diabetes mellitus (GDM)
5- Impaired glucose tolerance (IGT)
6- Impaired fasting glucose (IFG)

(Modified from the American Diabetes Association, 2007)

Type 2 Diabetes Mellitus:

Type 2 DM formerly called (non-insulin-dependent or adult-onset diabetes) comprises approximately 90% of all cases of diabetes. Patients have minimal symptoms, are not prone to ketosis, and are usually not dependent on insulin to prevent ketonuria. Type 2 diabetes in children and adolescents is an emerging significant problem ***(Lang et al., 2008).***

There are at least two major identified pathological defects in patients with T2DM, one is a decreased ability of insulin to act on the peripheral tissue; this is called insulin resistance and is thought to be the primary underlying pathological process. The other is β-cell dysfunction, which is an inability of the pancreas to produce sufficient insulin to compensate for the insulin resistance; thus there is a relative deficiency of insulin early in the disease and absolute insulin deficiency late in the disease ***(American Diabetes Association, 2009).***

Despite the lack of consensus, it is clear that type 2 DM is an extremely heterogeneous disease and no single cause is adequate to explain the progression from normal glucose tolerance to diabetes. The fundamental molecular defects in insulin resistance and insulin secretion result from a combination of environmental (as physical inactivity, sedentary lifestyle, generous consumption of alcohol and stress) and genetic factors, Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of DM, figure 2 ***(Sacks and McDonald, 2006; Abdulfatai et al., 2012; Ozougwu et al., 2013).***

Figure (2): Pathophysiology of type 2 DM ***(Sacks and McDonald, 2006).***

(D) Complications of Diabetes:

The complications of diabetes are classified into acute and chronic complications according to their onset, *table (2)* ***(Weiss and Sumpio, 2006).***

Table (2): Classification of diabetic complications.

1- Acute Complications:
a- Diabetic ketoacidosis.
b- Hyperglycemic hyperosmolar non ketotic coma.
c- Hypoglycemia.
d- Lactic acidosis.
2-Chronic Complications:
a- Microvascular complications.
i- Diabetic retinopathy.
ii- Diabetic nephropathy.
iii- Diabetic neuropathy.
b- Macrovascular complications.
i- Atherosclerosis.
ii- Coronary artery disease.
iii- Diabetic foot.
iv- Stroke.
v- Peripheral vascular disease.
vi- Diabetic myonecrosis.

(Modified from Weiss and Sumpio, 2006)

(1) Acute Complications:

(a) Diabetic ketoacidosis (DKA):

Pathophysiology:

DKA is a complex metabolic disorder state characterized by hyperglycemia, acidosis, and ketonuria. DKA usually occurs as a consequence of absolute or relative insulin deficiency that is accompanied by an increase in counter regulatory hormones (i.e., glucagon, cortisol, growth hormone, epinephrine). This hormonal imbalance enhances hepatic gluconeogenesis, glycogenolysis, and

lipolysis resulting in severe hyperglycemia and increase serum free fatty acids (figure 3) ***(Lin et al., 2006).***

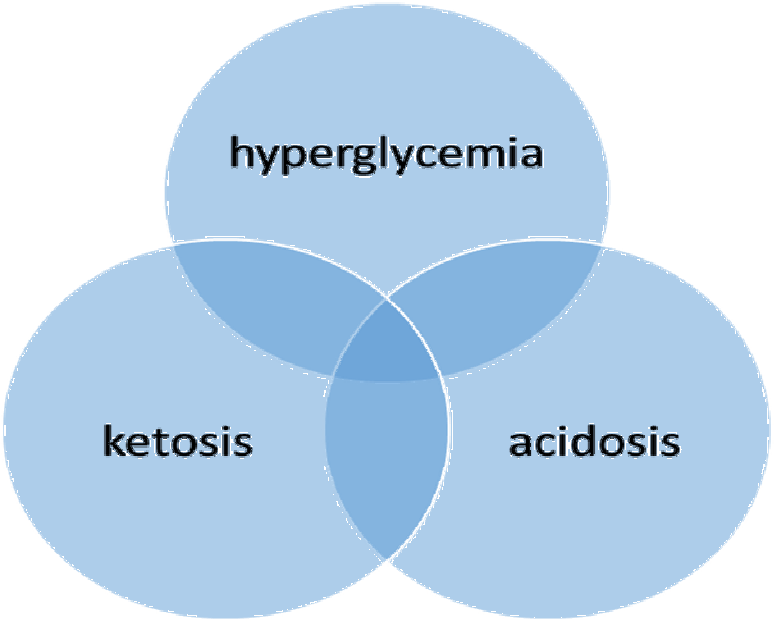

Figure (3): Pathophysilogy of diabetic ketoacidosis.

b) Hyperglycemic hyperosmolar state (HHS):

Hyperglycemic hyperosmolar state is the initial presentation of DM for 30-40% of patients. Most cases of HHS occur in patients with type 2 DM. This condition could be manifested during acute febrile illness or in conditions associated with elevation of counter regulatory hormones as (acromegaly, glucocorticoids use, or increase catecholamine in stress states) ***(Stoner, 2008).***

(c) Hypoglycemia:

Hypoglycemia is a blood glucose concentration below the fasting value, but it is difficult to define a specific limit. The most widely suggested cutoff is 50 mg/dL, but some authors suggest 60 mg/dL ***(Lindon et al., 2008).*** In patients with diabetes, this may be caused by several factors, the commonest cause is the side effects of drugs used for the treatment of diabetes. Other factors include alcohol, severe hepatic dysfunction, insulinoma, chronic renal failure. The patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system. Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death ***(Gispen and Biessels, 2008).***

(d) Lactic acidosis (LA):

Lactic acidosis consists of elevated lactic acid with acidosis (pH≤7.3) and without ketosis. Approximately half of the reported cases of LA have occurred in patients with DM. The usual precipitating factors for LA are hypoxia and some medications, such as a biguanide ***(Yang et al., 2009).***

(2) Chronic Complications:

Most diabetic complications arise from damage to blood vessels. Those more specific to diabetes affecting the retina, kidney and nervous system are termed microvascular giving rise to retinopathy, nephropathy and neuropathy, respectively.

On the other hand, those arising from accelerated atherosclerosis particularly affecting the coronary, carotid and femoral arteries and are termed macrovascular complications ***(Canani et al., 2008).***

(a) Microvascular complications:

Increase activation of the polyol pathway by increasing blood glucose level, increases the NADH / NAD+ ratio. This increases the activation of protein kinase C (PKC) and reduces activity of glyceraldehyde 3-phosphate dehydrogenase which converts glyceraldehyde 3 phosphate to pyruvate. Excess glyceraldehyde induces non enzymic protein glycation. This alters the structure and function of extracellular matrix proteins e.g. basement membrane of blood vessels and induces interactions with intracellular receptors which stimulate changes in the endothelial surface through release of cytokines and growth factors ***(Gappay, 2009).***

i) Diabetic retinopathy*:*

It is associated with growth of friable new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness ***(Wong et al., 2008)***.

ii) Diabetic neuropathy:

It is defined as abnormal or decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands. When

combined with damaged blood vessels this can lead to diabetic foot. Other forms of diabetic neuropathy may present as mononeuritis, autonomic neuropathy or diabetic amyotrophy which is muscle weakness due to neuropathy ***(King, 2008).***

iii) Diabetic nephropathy:

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/day or >200 μg/min) that is confirmed on at least two occasions 3-6 months apart, with declined glomerular filtration rate (GFR), and elevated arterial blood pressure ***(Kostadaras, 2009).***

(b) Macrovascular Complications:

The atheromatous plaque formations which are the main initiator of diabetic macrovascular disease are not different from those in non-diabetics but they tend to be more common and more extensive. The processes through which diabetes is thought to induce atherosclerosis in (carotids, femoral and coronaries) are attributed to the hyperglycemic effects on endothelial cell structure, platelet adhesion and stimulatory factors in plaque formation ***(Nabipour, 2003).***

E- Diagnosis of Diabetes Mellitus:

1- Clinical Diagnosis:

Diagnosis of diabetes is usually discovered by rapid onset of symptoms as polyuria, polydepsia and weight loss. The patient may be presented with complications at the time of

diagnosis as neuropathy, nephropathy, leg ulcer or diabetic ketoacidosis ***(Lyssenko, 2008).***

2- Laboratory Diagnosis:

(a) Screening recommendations:

The ADA guidelines recommended that all individuals of any age who are overweight or obese (BMI ≥25 kg/m2) and who have one or more additional risk factors for diabetes as (hypertention, dyslipidaemia, history of vascular disease, positive family history of diabetes in a 1st-degree relative, IGT (impaired glucose tolerance) or IFG (impaired fasting glucose) on previous testing and sedentary life style) should be tested for diabetes. In others without these risk factors, testing should begin at age 45 years ***(Erickson et al., 2012).***

(b) Guidelines of diabetes diagnosis:

In 2012, the ADA announced the new guidelines for diagnosis of diabetes, *table (3)*.