INTRODUCTION

Strict glycemic control in patients with diabetes decreases the incidence of diabetic complications, which can determine the quality of life and prognosis of such patients. Intensive treatment with insulin or oral hypoglycemic agents has been established to delay the onset and slow the progression of diabetic microangiopathy in the patients with types 1 diabetes and type 2 diabetes. Clinical evidence has suggested the favorable effects of strict glycemic control on cardiovascular disease, a main cause of death in patients with diabetes ***(Inaba et al., 2007)***.

The importance of accurately assessing glycemic status in the management of diabetic patients cannot be overemphasized. Various parameters including fasting glucose level, postprandial glucose level, and glycated hemoglobin are used to great effect in determining the glycemic status of patients, and their clinical significance has already been proven through numerous epidemiological and clinical studies. Although these three glycemic indices provide useful information, in several cases these indices alone are inadequate ***(Kim and Lee, 2012)***.

For example, appropriately monitoring glycated hemoglobin level is difficult in the setting of an abnormal hemoglobin metabolic pathway, such as anemia, decreased

renal function, and gestational diabetes. Furthermore, since glycated hemoglobin reflects a change in glycemic status over a period of 2 to 3 months (that reflects 120 day life span of erythrocytes), it is inappropriate in monitoring the therapeutic effects in earlier stages in the management of diabetes. Such limitations act as obstacles in the use of glycated hemoglobin levels in clinical settings ***(Kim et al., 2012).***

It has been reported that strict glycemic control, as indicated by lower glycated hemoglobin (HbA1c) levels, has beneficial effects on the prognosis of patients who have diabetes with chronic kidney disease and undergo regular hemodialysis (HD). However, some reports indicate that HbA1c might not provide a relevant assay for glycemic control in HD patients (false low results). This is because HbA1c values are influenced significantly in HD patients by either shortening of the life span of erythrocytes, periodic blood sampling, residual blood in dialysis circuit, mechanical hemolysis, recent transfusion, iron deficiency, metabolic acidosis or the changing proportion of young to old erythrocytes by erythropoietin use ***(Inaba et al., 2007)***.

Serum glycated albumin (GA) was hypothesized to be an alternative marker for glycemic control in patients with diabetes. It is not affected by changes in the survival time of erythrocytes in the case of type 2 diabetes with hemoglobinopathy ***(Inaba et al., 2007)***.

The half-life of albumin is approximately 15 days, and GA level is believed to reflect the glycemic change over a 2-week period. Therefore, GA can be useful in evaluating the therapeutic effect of recently substituted hypoglycemic agents at an early stage ***(Koga and Kasayama, 2010)***.

In contrast to HbA1c, glycated albumin itself is strongly involved in the development of major diabetic complications, including arterial stiffening, peripheral vascular calcification, nephropathy, neuropathy, retinopathy, coronary artery disease and Alzheimer's disease. This is because GA is a precursor of advanced glycation end products (AGEs) ***(Kim and Lee, 2012)***.

Initially, the utility of GA was viewed as an adjunct to HbA1c for diabetes management but is gaining popularity in month-based management of diabetes and diabetes-associated pathologies, such as hemolytic anemia, end stage renal disease (ESRD), and iron deficiency, as well as pregnancy. In addition, albumin is a pivotal antioxidant in human serum. Therefore, glycation and accompanied oxidation of albumin leads to a loss of antioxidant activity and generates an atherogenic protein in diabetes. This could explain why higher level GA related with vascular complication and diminished insulin secretory function ***(Kim and Lee, 2012).***

Glycated albumin was initially evaluated by high performance liquid chromatography (HPLC). A new enzymatic

method of determining GA was developed, and was confirmed to have sufficient accuracy and good correlation with the conventional HPLC method. Since this method can be employed in an auto-analyzer, rapid and multiple determinants can be performed ***(Takahashi et al., 2007)***.

AIM OF THE WORK

The aim of this study was to assess the utility of glycated albumin as a substitute to HbA1c quantification for the accurate assessment of glycemic control in patients with end stage renal disease. In addition, to attempt to develop an estimating equation for investigating the relationship between glycated albumin and average glucose levels.

Chapter 1

DIABETES MELLITUS

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate metabolism characterized by hyperglycemia resulting from defects in either insulin secretion, insulin action or both. The chronic hyperglycemic state of diabetes is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels ***(American Diabetes Association, 2013)***.

A) Epidemiology of Diabetes Mellitus:

The World Health Organization (WHO) estimated that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death ***(WHO, 2014).*** In **2014, the International Diabetes Federation (IDF)** estimated that diabetes resulted in 4.9 million deaths ***(https://en.wikipedia.org /wiki/Diabetes_mellitus#Epidemiology).***

It is estimated that nearly 387 million people in the world suffer from diabetes. Its prevalence exceeds 8.3% and gradually increases. Most people with diabetes live in low- and middle-income countries ***(Zylca et al., 2015).***

B) Classification of Diabetes Mellitus:

The American Diabetes Association (ADA) (2013) established a work group to re-examine the old classification of diabetes mellitus and published the revised classification based on the origin and pathogenesis of diabetes in 1997 (Table 1).

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)

(American Diabetes Association, 2013)

C) Complications of Diabetes Mellitus:

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

Table (2): Complications of DM

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

(Weiss and Sumpio, 2006)

D) Diagnosis of Diabetes Mellitus:

1- Clinical Diagnosis:

Diagnosis of diabetes is usually prompted by rapid onset symptoms of 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 ***(ADA, 2013).***

The diagnosis of diabetes mellitus depends solely on demonstration of hyperglycemia. For type1 diabetes, the diagnosis is usually easy because hyperglycemia appears abruptly, while in type2 it is difficult because hyperglycemia is often not severe enough. It is estimated that 30% of the affected patients are undiagnosed with an estimated lag of 5 to 7 years between the onset of diabetes and diagnosis, but the risk of complications makes it very important to identify patients early ***(Seino et al., 2010).***

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 [hypertension, dyslipidemia, 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 those without these risk factors, testing should begin at age 45 years ***(Erickson et al., 2012)*.**

(b) Guidelines for diagnosis of DM:

In 2016, the ADA announced the new guidelines for diagnosis of diabetes (table 3).

Table (3): The ADA Recommendation for Diabetes Diagnosis

Any one of the following is diagnostic:

1. Classic symptoms of diabetes and casual plasma glucose concentration ≥200 mg/dl.
2. Fasting plasma glucose ≥126mg/dl.
3. 2-hour postprandial plasma glucose concentration ≥200 mg/dL during the OGTT.
4. Hemoglobin A1c (HbA_{1c}) ≥ 6.5%.

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeating the same test on a different day.

Impaired Fasting Glucose

Fasting plasma glucose between 100 and 125 mg/dl.

Impaired Glucose Tolerance

Two criteria must be met:

1. Fasting plasma glucose ≤126 mg/dl.
2. 2-hour OGTT plasma glucose concentration is between 140 and 199 mg/dl.

If any of these test results occurs, testing should be repeated on a different day to confirm diagnosis.

(American Diabetes Association, 2016)

(c) Monitoring patient compliance to treatment:

i. Self monitoring of blood glucose (SMBG):

The goal of SMBG is to collect detailed information about blood glucose levels at many time points to enable maintenance of a more constant glucose level by more precise regimens ***(American Diabetes Association, 2013).***

ii. Glycated hemoglobin (HbA1c):

Glycation is the non enzymatic addition of sugar residue to the amino-groups of proteins. In the normal 120-day life span of the red blood cell, glucose molecules join hemoglobin, forming glycated hemoglobin. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation over the previous four weeks to three months ***(Geistanger et al., 2008).***

The reference range of HbA1c is <5.7% in healthy persons and about 5.7% - 6.4% in a prediabetic patient. However in a diabetic person the goal is to keep HbA1c level at or below 6.5 - 7% as the ADA recommended ***(American Diabetes Association, 2016).***

iii. Fructosamine:

Fructosamine is formed by a chemical reaction of glucose with plasma protein, mainly albumin, and reflects glucose control in the previous 1-3 weeks. This assay, therefore, may show a change in control before HbA1c and often is helpful when applying intensive treatment and in short-term clinical trials ***(Danese et al., 2015).***

Chapter 2

DIABETIC NEPHROPATHY

A) Introduction:

Diabetic nephropathy (DN) comprises a triad of albuminuria (≥ 30 mg/day or ≥ 20 μg/min) confirmed on at least two occasions 3-6 months apart, permanent and irreversible decrease in glomerular filtration rate (GFR), and arterial hypertension. This triad heralds the onset of a dramatically increased risk of death, frequently as a result of premature cardiovascular disease ***(Eboh and Chowdhury, 2015)***.

B) Stages:

There are five stages in the development of diabetic nephropathy according to the National Kidney Foundation-Disease Outcomes Quality Initiative (K/DOQI) classification ***(Fiseha, et al., 2014).***

1. **Stage I: Hypertrophic Hyper-filtration**; in this stage, GFR is either normal or increased. It lasts approximately five years from the onset of the disease. The size of the kidneys is increased by approximately 20% and renal plasma flow is increased by 10%-15%, while albuminuria and blood pressure remains within the normal range.

2. **Stage II**: **The Quiet Stage**; this stage starts approximately two years after the onset of the disease and is characterized by kidney damage with basement membrane thickening and mesangial proliferation. There are still no clinical signs of the disease. GFR returns to normal values. Many patients remain in this stage until the end of their life.

3. **Stage III: The Microalbuminuria Stage (albumin 30-300 mg/day) or Initial Nephropathy**; this is the first clinically detectable sign of glomerular damage. It usually occurs five to ten years after the onset of the disease. Blood pressure may be increased or normal. Approximately 40% of patients reach this stage.

4. **Stage IV: Chronic Kidney Failure (CKF) is The Irreversible Stage**; Proteinuria develops (albumin >300 mg/day), GFR decreases below 60 mL/min, and blood pressure increases above normal values.

5. **Stage V: Terminal Kidney Failure (TKF) (GFR <15 mL/min);** approximately 50% of the patients with TKF require kidney replacement therapy (peritoneal dialysis, hemodialysis, kidney transplantation).

(Fiseha et al., 2014)

C) Pathogenesis:

Pathogenesis of diabetic nephropathy is very complicated and results from the interaction of hemodynamic and metabolic factors ***(Zylka et al., 2015).***

1. **Glomerular Hyperfiltration**; Blocking of the renin-angiotensin system and antagonizing the profibrotic effects of angiotensin II ***(Cachat et al., 2015).***

2. **Hormones;** Infusion of somatostatin analogues (octreotide) especially insulin-like growth factor-1 (IGF-1) partly lead to the decrease in hyperfiltration and kidney size ***(Cherney et al., 2005).***

3. **Sorbitol;** The enzyme aldose reductase converts intracellular glucose to sorbitol, which remains in the cell ***(Grewal et al., 2015).***

4. **Glycation End-Products;** Part of the excess glucose in chronic hyperglycemia binds to free amino acids of circulating or tissue proteins. This non-enzymatic process produces reversible early glycation products, and later, irreversible advanced glycation end products (AGEs), which accumulate in the tissues and contribute to the development of microvascular complications of DM ***(Schrijvers et al., 2004).***

5. **Hyperglycemia;** leading to mesangial cell proliferation, matrix expansion, and glycosylation of glomerular proteins ***(Zylka et al., 2015).***

6. **Heparanase Expression;** the reduction in heparin sulfate on the surface of endothelial cell changes the negative charge of glycocalyx and consequently increases albumin permeability of the glomerular filtration membrane ***(Van Den Hoven et al., 2006).***

7. **Reactive Oxygen Species (ROS);** it activate all important pathogenetic mechanisms, such as increased production of AGEs, increased glucose entry into the polyol pathway, and protein kinase C (PKC) activation. In addition, ROS directly damage endothelial glycocalyx, which leads to albuminuria without the concurrent damage to the glomerular basement membrane itself ***(Dronavalli et al., 2008).***

8. **Prorenin;** it binds to a specific tissue receptor, leading to the activation of the signal pathway of mitogen-activating protein kinases (MAPK), which potentiate the development of kidney damage ***(Nguyen, 2006).***

9. **Cytokines and Growth Factors;** Hyperglycemia stimulates increased expression of different growth factors and activation of cytokines, which overall contributes to further kidney damage ***(Eboh and Chowdhury, 2015)***.