INTRODUCTION

Diabetes, a disorder of glucose homeostasis, is an increasingly prevalent disease worldwide. Two distinct subtypes are recognized: autoimmune diabetes (Type 1 diabetes, T1D) typically affecting the young and associated with destruction of β-cells and non-immune diabetes (Type 2 diabetes, T2D) ***(Chia et al., 2012)***.

Type 2 diabetes is a global health problem characterized by a defect in insulin secretion and/or a decrease in sensitivity to insulin resulting in an increase in blood glucose levels. It is strongly associated with obesity, with a low-grade inflammation driven by long-term nutrient excess ***(Shu et al., 2012)***.

Studies have shown that inflammation, and more specifically pro-inflammatory cytokines, play a determinant role in the development of microvascular diabetic complications (diabetic neuropathy, nephropathy and retinopathy) ***(Navarro and Mora, 2005).***

Regulatory T cells (Treg) play a fundamental role in the modulation of body weight, adipocyte hypertrophy, insulin resistance, glucose tolerance and T2D progression in mice and humans ***(Winer et al., 2009)***. Treg cells have various mechanisms to suppress the inflammatory response; cytokine secretion, cell death, surface receptors

and modulation of the microenvironment ***(Schmetterer et al., 2012).*** In particular, an important role for adenosine in mediating the immunosuppressive properties of Treg cells has been reported. Treg cells metabolize adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in the extracellular space to adenosine, which inhibits the immune system locally ***(Borsellino et al., 2007; Deaglio et al., 2007).***

Under basal conditions extracellular concentrations of ATP are maintained at low levels. Endogenous regulation of ATP concentration is mediated by ectoenzymes: the family of ectonucleotidases (E NTPDases) and ecto-5'-nucelotidase (CD73) ***(Robson et al., 2006).*** The CD39 (NTPDase-1) and CD73 are two cell-surface ecto-enzymes that dephosphorylate ATP into its metabolites, ADP, adenosine monophosphate (AMP) and adenosine, in a tightly regulated process ***(Beavis et al., 2012)***. Adenosine induces inhibitory and anti-proliferative effects through binding to its four receptors: A1, A2A, A2B and A3 ***(Regateiro et al., 2013)***.

The CD39 and CD73 have been widely considered vital in the generation of immunosuppressive microenvironments through adenosine production**.** In accordance with this idea, CD39 and CD73 ectonucleotidases are reported as active players in several

diseases, such as cancer, autoimmunity, allergy, and ischemia–reperfusion injury ***(Antonioli et al., 2012).*** They are expressed at different levels in a variety of tissues, including the heart, placenta, lung, liver, colon, brain and kidney ***(Thompson et al., 2004).*** Human Treg cells have been reported to express CD39 on their surface, with intracellular CD73 expression. It has been demonstrated that CD39 and CD73 ectonucleotidases are key in adenosine production by Treg cells and therefore are considered to be part of their immunosuppressive arsenal ***(Deaglio et al., 2007)***. Some reports have shown that in addition to Treg cells, other T-cell subsets, neutrophils and B cells also express the CD39 and/ or CD73 ectonucleotidases ***(Chen et al., 2006)***.

Aim of the Work

This work aims to determine the expression of CD73 and CD39 on different peripheral blood leukocytes including Treg cells to uncover their inflammatory modulation role in T2D subjects in comparison to normal control ones. The level of their expression will be correlated with various anthropometric and biochemical parameters in diabetic patients.

DIABETES MELLITUS

Diabetes was first documented by the Egyptians and is characterised by weight loss and polyuria. However, the Greek physician Aertaeus is the one who gave it the term diabetes mellitus (DM), diabetes in Greek means "to pass through" and mellitus is the Latin word for “honey”. Diabetes is an outstanding cause of chronic illness and premature mortality. Its mortality rate is more than HIV-AIDS with nearly 1 death every 10 seconds ***(Kaul et al., 2012)***.

A. Definition:

Diabetes mellitus is a disorder of glucose metabolism characterized by a state of chronic hyperglycemia. It is caused by deficiency in insulin secretion, abnormal insulin action or both. It is a systemic disorder causing damage and dysfunction of multiple organs ***(Rother, 2007)***.

B. Epidemiology:

In the year of 2000, as indicated by the World Health Organization, no less than 171 million individuals, or 2-8% of the world population have DM ***(Wild et al., 2004)***.

In the year of 2008, there were around 24 million individuals with diabetes in the United States alone, from those 5.7 million individuals stay undiscovered. As indicated by the American Diabetes Association, around 18.3% (8.6 million) of Americans older than 60 years old have diabetes. As DM is more common in old age and with

the fact that the elderly population is increasing, the number of old diabetic patients is growing ***(American Diabetes Association, 2008)***.

In the year of 2010, diabetic patients constituted 6.4% of the world adult population (285 million individual) ***(Shaw et al., 2010).*** In 2013, 382 million adults were diagnosed with diabetes worldwide. This number is expected to grow to 592 million in 2035 ***(International Diabetes Federation, 2015).***

The International Diabetes Federation (IDF) listed Egypt among the world top 10 countries in the number of patients with diabetes. It is expected that the number of patients with diabetes in the Middle East and North Africa region to grow by 96% from year 2013 to 2035 or from 34.6 million to 67.9 million. In Egypt, the prevalence of diabetes is around 15.56% among adults between 20 and 79 years of age, with an annual death of 86,478 related to diabetes. In 2013, the IDF estimated that 7.5 million individuals have diabetes and around 2.2 million have prediabetes in Egypt. Furthermore, reports indicate that 43% of patients with diabetes and most patients with prediabetes in Egypt are likely undiagnosed. It is alarming that diabetes prevalence in Egypt has increased rapidly within a relatively short period from approximately 4.4 million in 2007 to 7.5 million in 2013. It is expected this number will jump up to 13.1 million by 2035 ***(International Diabetes Federation, 2015).***

Diabetic patients are found all over the world, but the more the country is developed the more the prevalence of diabetes especially T2D. By 2030, it is expected that most of the patients will be found in Asia and Africa. The expansion in occurrence in the developing countries is most probably due to urbanization and the way life is changing in these countries, in particular the "Western-Style" diet. This has proposed the environmental factor in diabetes, yet there is still small comprehension of the mechanism(s) at present, however there is much theory, some of it most compellingly introduced ***(Wild et al., 2004)***.

C. Classification of Diabetes:

Table 1: Etiological classification of diabetes mellitus

(a) Type 1 Diabetes (T1D):
i- Immune mediated. ii- Idiopathic.
(b) Type 2 Diabetes (T2D).
(c) Other Specific Types of Diabetes: i- Genetic defects of islet ß-cell function. ii- Genetic defects of insulin action. iii- Diseases of the exocrine pancreas. iv- Endocrinopathies. v- Drug- or chemical- induced diabetes. vi- Infections. vii- Uncommon forms of diabetes. viii-Other genetic syndromes.
(d) Gestational Diabetes Mellitus (GDM).

(American Diabetes Association, 2010)

a) Type 1 Diabetes Mellitus:

Type 1 diabetes (T1D), formerly called insulin dependent diabetes or juvenile onset diabetes is an autoimmune disorder caused by activated CD4+, CD8+ T cells and macrophages which invade pancreatic islets causing beta cell destruction ***(Phillips et al., 2009).*** T1D occurs mostly in young ages (<35 years old). Various genetic and environmental factors make individuals more prone to this type of diabetes. Studies showed that human leucocyte antigen (HLA) gene found on chromosome 6 is related to T1D. The HLA protein is a self-antigen found on the cell surface helping the immune system discriminating self and foreign agents. In T1D, defect in HLA proteins give rise to an autoimmune reaction causing the immune system to attack the beta cells ***(Gorodezky et al., 2006)***.

Some studies suggest that some viruses also trigger T1D**.** A significant number of viruses have been associated with T1D, including enteroviruses such as Coxsackievirus B ***(Hyöty and Taylor, 2002)***, but also rotavirus ***(Honeyman et al., 2000)***, mumps virus ***(Hyöty et al., 1988)***, and cytomegalovirus ***(Pak et al., 1988)***. The prime viral candidates for causing T1D in humans are enteroviruses ***(Hyöty et al., 1995)***

Another form of T1D is the idiopathic diabetes, where immunity has no role in its pathogenesis. Its

prevalence is less than that of autoimmune T1D and it affects more the people in Asia and Africa. Its cause is still unknown but patients with this type of T1D have no insulin production nor antibodies against the beta cells and they are susceptible to ketoacidosis ***(Harris et al., 1998)***.

b) Type 2 Diabetes:

Type 2 diabetes (T2D) previously known as non-insulin-dependent or adult-onset diabetes represents 90% of diabetic cases. Patients with T2D are not dependant on insulin, ketoacidosis is uncommon and have minor symptoms ***(Lang et al., 2008).***

There are two main pathological abnormalities in these patients. First, the insulin resistance which is a decline in the effect of insulin on the peripheral tissue, which is believed to be the primarily defect in T2D. Second, beta cell dysfunction which is a decrease in insulin synthesis in the pancrease making it unable to overcome the peripheral insulin resistance, thus there is a relative deficiency of insulin early in the disease and absolute insulin deficiency later on ***(Eberhart et al., 2004).*** T2D is a multifactorial disorder in which both insulin resistance and beta cell dysfunction are influenced by multiple environmental and genetic factors ***(Sacks and McDonald, 2006).***

Insulin resistance:

Insulin resistance is defined as a decline in the effect of normal levels of insulin on body cells, the condition is noted in both T2D patients and in obese non-diabetic individuals ***(Flier, 2002).***

The insulin resistance syndrome (also known as syndrome X or the metabolic syndrome) is a combination of clinical and laboratory findings: insulin resistance, hyperinsulinemia, obesity, dyslipidemia (high triglyceride and low HDL-cholesterol) and hypertension ***(Hill et al., 2005).***

Beta cell dysfunction:

Insulin resistance leads to increase in the needs for release of insulin by beta cells which in return causes progressive beta cell dysfunction, which leads to decline in insulin production in response to blood glucose (termed as selective glucose unresponsiveness). High levels of blood glucose make the beta cells more unresponsive to glucose (glucotoxicity). Beta cell dysfunction depends on both blood glucose level and duration of hyperglycemia. Restoring the blood glucose level to its normal levels resolves the problem ***(Bell and Polonsky, 2001)***.

Beta cell dysfunction is also caused by increased fatty acids in the serum. In T2D there is also a defect in the normal pulsatile release of insulin ***(Bell and Polonsky, 2001).***

Environmental factors:

Diet and exercise are important factors in the pathogenesis of T2D. There are studies relating obesity to T2D, as fat especially abdominal fats emit group of hormones known as adipokines which may cause glucose intolerance ***(Baillie, 2008).***

It was found that there is an inverse relationship between the physical activity level and risk for T2D, this protective effect of exercise is thought to be due to rise in insulin sensitivity in adipose tissue and skeletal muscles. Independently of both body weight and family history of diabetes, there is a decrease of 6% in the risk for T2D with every 500-kcal increase in daily energy expenditure ***(Mokdad et al., 2003).***

Genetic factors:

It is broadly recognized that hereditary variables add to the advancement of T2D. For instance, the concordance rate for T2D in identical twin is nearly 100%. An obese person with a family history of diabetes has a risk to develop T2D 10 times more than an obese person without family history of diabetes, but the mode of inheritance is unknown ***(Kahn et al., 2003).***

Risk factors for Type 2 Diabetes:

a- Overweight (Body mass index ≥ 25 kg/m²): About 80% of type 2 diabetic patients are obese and have sedentary life styles ***(Venables and Jeukendrup, 2009)***. It was found that high levels of circulating fatty acids cause decline in insulin signaling and glucose disposal rates ***(Belfort et al., 2005)***.

b- Habitual physical inactivity: Numerous forthcoming studies showed that individuals with daily physical activity have lower chances to develop T2D. Recently it was found that after an acute bout of exercise there is an increase in the mitochondrial oxidative capacity which in return causes decrease in the incidence of T2D ***(Venables and Jeukendrup, 2009)***.

c- Age ≥ 45 years: It is still uncertain whether the increase in insulin resistance with aging is due to increase in the abdominal fat or not. Actually increase in the abdominal fat leads to changes in the insulin action and dyslipidemia ***(Lovejoy et al, 2008)*.**

d- Positive family history of T2D: In clinical assessment of diabetic patients with T2D genetic information and family history is important ***(Lyssenko et al., 2008)***. Individuals with one diabetic parent has an 40% increased risk to develop T2D and 70% increase if both parents are diabetic ***(Majithia and Florez, 2009)***.

e- **Previously diagnosed impaired fasting glucose or impaired glucose tolerance:** Development of overt diabetes happens at a rate of 1% to 5% per year in these patients ***(Diabetes Epidemiology, 2003)***.

f- **History of gestational diabetes (GDM) or delivery of an overweight baby (weight > 4kg):** The risk for development of T2D in women with history of gestational diabetes is increased from 20 to 40% ***(Centers for Disease Control and Prevention, 2005).***

g- **Hypertension** (Blood pressure ≥140/90 mmHg in adults).

h- **High density lipoprotein-cholesterol (HDL-C**) ≤ **35mg/dl or triglycerides ≥ 250mg/dl** ***(American diabetes association, 2010)***.

c) Gestational Diabetes Mellitus:

Gestational diabetes mellitus (GDM) is defined as onset or first recognition of any glucose intolerance during pregnancy. The prevalence of GDM varies according to the criteria used to diagnose it and the population under study ***(Galtier, 2010).*** GDM occurs when β-cells cannot compensate for the increased levels of insulin resistance ***(Catalano, 1994).*** GDM predispose to long- and short-term complications to the mother and child ***(Bellamy et al., 2009)***. For the child, it includes development of T2D and obesity ***(Kim et al., 2011).*** For the mother, it includes cardiovascular complications and development of T2D ***(Hopmans et al., 2015).***

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) Hypoglycemia
- c) Hyperglycemic hyperosmolar non ketotic coma
- d) Lactic acidosis

2- Chronic Complications
- a) Microvascular complications
  - Diabetic retinopathy
  - Diabetic nephropathy
  - Diabetic neuropathy
- b) Macrovascular complications
  - Atherosclerosis
  - Coronary artery disease
  - Diabetic foot
  - Stroke
  - Peripheral vascular disease
  - Diabetic myonecrosis

(modified from Weiss and Sumpio, 2006).

E. Diagnostic Criteria:

The American Diabetes Association (ADA) proposed criteria for the diagnosis of diabetes which include any of the following: ***(Diagnosis and classification of diabetes mellitus, 2010)***

- A hemoglobin A1c (HbA1c) level of 6.5% or higher; the test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay, **or**

- A fasting plasma glucose (FPG) level of 126 mg/dL (7 mmol/L) or higher; fasting is defined as no caloric intake for at least 8 hours, **or**

- A 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), **or**

- A random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia (i.e. polyuria, polydipsia, polyphagia, weight loss) or hyperglycemic crisis.