INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease characterized by immune mediated β cell destruction and consequential insulin deficiency. This leads to metabolism disorders with chronic hyperglycemia as the main feature, which in turn causes exceeded production of Advanced Glycosylation end products which lead to macrophage activation, increased oxidative stress and production of inflammatory cytokines.

Chronic inflammation causes endothelial dysfunction which is the key event in the development of microvascular and macrovascular complications in diabetic patients ***(Maiti et al., 2007)***.

Microvascular complications increase morbidity and mortality of type 1 diabetes mellitus patients by causing increased incidence of blindness, terminal kidney failure, arthropathy, foot trauma, foot ulceration, infection and lower limb amputation.

Macrovascular complications are caused by cardiovascular, cerebrovascular and peripheral artery damage leading to heart attack, stroke and peripheral artery occlusion followed by increased incidence of lower limb amputation ***(Mitrovic et al., 2014).***

Patients with type 1 diabetes mellitus especially those who developed microvascular and macrovascular complications, have increased inflammatory activity expressed through elevated levels of inflammatory cytokines, mainly C reactive protein(CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and recently soluble urokinase plasminogen activator receptor (suPAR) ***(Chase et al., 2004; Devaraj et al., 2006).***

Soluble urokinase-type plasminogen activator receptor (SuPAR), is the soluble form of urokinase plasminogen activator receptor (uPAR). UPAR is a membrane bound receptor for uPA, otherwise known as urokinase. SuPAR results from the cleavage and release of membrane-bound uPAR ***(Thunø et al., 2009).***

SuPAR, a marker of inflammation and endothelial dysfunction released from inflammatory cells and has a role in inflammation, thrombosis and cell proliferation. Under physiological conditions suPAR, the membrane bound form of uPAR, maintains tissue function through regulation of cell proliferation, adhesion, migration and proteolysis. However during inflammation, recruitment and expression of uPAR/suPAR are upregulated in endothelial cells, fibroblasts, macrophages and glomerular cells ***(Blasi, 1997; Ossowski, Aguirre, 2000).***

In renal cells, suPAR is associated with podocyte and glomerular basal cell membrane damage leading to proteinuria,

thus increased plasma levels of suPAR are indicative of renal damage. It has been shown that suPAR is the cause of glomerular focal sclerosis apparently through podocyte damage ***(Wei et al., 2011)*** and uPAR contributes to renal ischaemia and cellular apoptosis in a mouse allograft model and inhibition of uPAR has been shown to decrease proteinuria in mice with glomerular disease ***(Zhang et al., 2012).***

Extra-renal production of suPAR from immune cells may also contribute to the plasma level of suPAR and represent inflammation in other tissues. Inflammation is important in the development of cardiovascular disease ***(Haverkate et al., 1997),*** and as suPAR seems to be a stable marker of the immune activation and inflammatory status of the individual, this may explain why suPAR is a risk marker not only for cardiovascular disease but also for the outcome of infectious diseases, cancer and for all causes of mortality ***(Eugen-Olsen et al., 2010).***

AIM OF THE WORK

Evaluation of Soluble Urokinase Plasminogen Activator Receptor (suPAR) as a marker of inflammation and endothelial dysfunction in patients with Type 1 Diabetes Mellitus and to assess its relation to various clinical and biochemical markers of diabetes as well as Diabetes related Vascular Complications.

DIABETES MELLITUS

Diabetes Mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action, or both ***(International Society of Pediatric and Adolescent Diabetes ISPAD, 2014).*** The chronic hyperglycemia of diabetes is associated with relatively specific long term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD) ***(ADA, 2012)***.

Diabetes mellitus is not a simple disease, but it is a heterogonous group of disorders in which there are distinct genetic patterns of inheritance as well as separate etiologic and patho-physiologic mechanisms all leading to impairment of glucose metabolism ***(Dabelea et al., 2011)***.

Classification of diabetes

The type of diabetes assigned to a young person at diagnosis is typically based on their characteristics at presentation, however, increasingly the ability to make a clinical diagnosis has been hampered by factors including the increasing prevalence of overweight in young people with type 1 diabetes ***(Islam et al., 2014; Kapellen et al., 2014)*** and the presence of diabetic ketoacidosis (DKA) in some young people at diagnosis of type 2 diabetes ***(Dabelea et al., 2011).***

In addition, the presentation of a familial form of mild diabetes during adolescence should raise the suspicion of

monogenic diabetes, which accounts for 1–4% of pediatric diabetes cases ***(Irgens et al., 2013; Pihoker et al., 2013).***

Table (1): Etiological classification of diabetes mellitus:

I. Type I-DM: (B-cell destruction, usually leading to absolute insulin deficiency) A. Immune mediated. B. Idiopathic.	**II. Type II-DM:** (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance).
III. Other specific types	
A. Genetic defects of β-cell function: **1.** MODY3 (Chromosome 12, HNF1α) **2.** MODY1 (Chromosome 20 HNF4α) **3.** MODY2 (Chromosome7glucokinase) **4.** Other very rare forms of MODY (e.g. MODY4: Chromosome 13, insulin Promoter factor-1, MODY6: Chromosome9, carboxyl ester lipase) **5.** Transient neonatal diabetes **6.** Permanent neonatal diabetes **7.** Mitochondrial DNA **8.** Others	**B. Genetic defect in insulin action** **1.** Type A insulin resistance **2.** Leprechaunism **3.** Rabson-Mendenhall syndrome **4.** Others
C.Diseases of exocrine pancreas **1.** Pancreatitis **2.** Truama/ pancreatectomy **3.** Neoplasia **4.** Cystic fibrosis **5.** Haemochromatosis **6.** Fibrocalculous pancreatopathy **7.** Others	**D. Endocrinopathies** **1.** Acromegaly **2.** Cushing's syndrome **3.** Glucagonoma **4.** Pheochromocytoma **5.** Hyperthyroidism **6.** Somatostatinoma **7.** Aldosteronoma **8.** Others
E. Drugs or chemical- induced **1.** Vacor **2.** Pentamidine **3.** Nictotinic acid **4.** Glucocorticoids **5.**Thyroid hormone **6.** Diazoxide **7.** B-adrenegic agonist **8.** Thiazides **9.** Dilantin **10.** γ-interferon **11.** Others	**F. Infections** **1.** Congenital rubella **2.** Cytomegalo virus **3.** Others
G. Uncommon forms of immune-mediated Diabetes **1.**"Stiff-man" syndrome **2.** Anti-insulin receptor antibodies **3.** Others	**H. Other genetic syndromes sometimes associated with diabetes** **1.** Down syndrome **2.** Klinfelter syndrome **3.** Turner syndrome **4.** Wolfram syndrome **5.** Friedreich's ataxia **6.** Huntington's chorea **7.** Laurence-Moon-Biedle syndrome **8.** Myotonic dystrophy **9.** Porphyria **10.** Prader-Willi syndrome **11.** Others
IV. Gestational diabetes.	

MODY: Maturity onset diabetes of the young, **HNF-4α:** Hepatocyte Nuclear Factor, **NeuroD1:** Neurogenic differentiation.

(ISPAD, 2014)

The differentiation between type 1, type 2, monogenic, and other forms of diabetes has important implications for both therapeutic decisions and educational approaches. Diagnostic tools, which may assist in confirming the diabetes type, include:

- Diabetes-associated autoantibodies: the presence of GAD, IA2, IAA, and/or ZnT8 confirms the diagnosis of type 1 diabetes, as one and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected ***(Watkins et al., 2014).***

- An elevated fasting C-peptide level can distinguish young people with non-autoimmune, insulin resistant type 2 diabetes from type 1 diabetes ***(Dabelea et al., 2011).***

However, as there is considerable overlap in insulin or C-peptide measurements between type 1 and type 2 diabetes in the first year after diagnosis, C-peptide measurements are not recommended in the acute phase. If patients are insulin treated, measuring C-peptide when the glucose is sufficiently high (>8 mmol/L) to stimulate C peptide will detect if endogenous insulin secretion is still present. This is rare beyond the remission phase (2–3 yr) in children with type 1 diabetes ***(Dabelea et al., 2011).***

The possibility of monogenic diabetes should be considered in the child who has no autoantibodies and:

- An autosomal dominant family history of diabetes;
- Diabetes diagnosed in the first 6 months of life;
- Mild fasting hyperglycemia [5.5–8.5 mmol (100–150 mg/dL)], which does not progress, especially if young, non-obese, and asymptomatic;
- Associated conditions such as deafness, optic atrophy, or syndromic features; and
- A history of exposure to drugs known to be toxic to β cells or cause insulin resistance.

Type 1 Diabetes Mellitus

Type 1 diabetes is a disorder that arises following the autoimmune destruction of insulin-producing pancreatic b cells ***(Atkinson, 2001; Bluestone et al., 2010)***. The disease is most often diagnosed in children and adolescents, usually presenting with a classic trio of symptoms (i.e., polydypsia, polyphagia, polyuria) alongside of overt hyperglycemia, positing the immediate need for exogenous insulin replacement-a medicinal introduction to the disorder whose therapeutic practice lasts a lifetime ***(Thunandera et al., 2008).***

Incidence and Prevalence of Type 1 diabetes mellitus in Egypt

The incidence of type 1 diabetes mellitus (T1DM) is increasing in all population at a rate of approximately 3% per year and the onset of the condition is occurring at younger age. Its incidence varies dramatically between populations and even within the same population, much of this variation is due to genetic defect. Among Eastern Mediterranean and Middle Eastern countries, the largest contribution to the total number of estimated childhood T1DM cases comes from Egypt which accounts for about a quarter of the region's total. An Egyptian study of incidence and prevalence of T1DM in children and adolescents in four Egyptian Governorates (Fayoum, Minofeya, North Sainai and Sues) was held by ***Salem et al. (2007)***, showing a prevalence rate of 0.7/1000 and an incidence rate of 4.01/100.000.

- **Age**:

T1DM accounts for more than 90% of childhood and adolescent diabetes ***(ISPAD, 2009),*** with peaks of presentation occuring in two age groups:

- ***First peak***: At 5-7 years which corresponds to increased exposure to infectious agents with the start of school time.
- ***Second peak*:** At time of puberty which corresponds to the pubertal growth spurt induced with gonadal steroids and

increased growth hormone secretion, both antagonize insulin hormone ***(Alemzadeh and Wyatt, 2008).***

- **Sex and socioeconomic class**

Girls and boys are almost equally affected. There is no apparent correlation with socioeconomic status ***(Alemzadeh and Wyatt, 2008).***

- **Seasonal variations:**

Seasonal variation in the presentation of new cases is well described with the beginning of winter months ***(Ismail et al., 2008).***

- **Place:**

Type 1 diabetes incidence varies greatly between different countries, within countries, and between different ethnic populations, with the highest incidence rates observed in Finland, Northern Europe, and Canada.

- **Classification of type 1 diabetes**

Type 1 diabetes, may be subdivided, in two groups from the etiological point of view: Autoimmune and Idiopathic.

- Type 1A, the autoimmune type, which is polygenic and it is the most frequent type of this disease, corresponding to approximately 80-90% of all T1DM cases ***(Daneman, 2006).***

- Type 1B, the idiopathic type, has all the clinical features of type 1A, but the autoimmune component is not detected ***(American Diabetes association, 2008).***

- **Etiology and Pathogenesis*:***

Type 1 diabetes is a multi-factorial autoimmune disease with both environmental and genetic susceptibility ***(Cruz et al., 2004).***

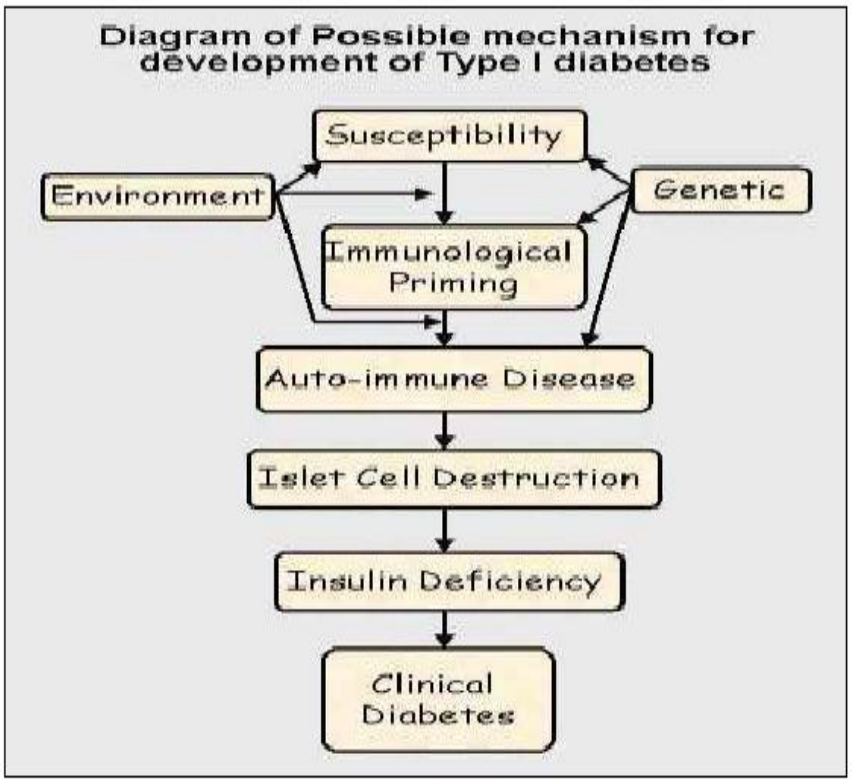

Figure (1): Possible mechanism for development of T1DM ***(Lamb, 2011).***

The etiology of type 1 diabetes remains poorly understood, but it is likely that an environmental factor triggers an autoimmune process in a predisposed individual.

***A) Genetic predisposition*:**

Approximately 10% of cases of type 1 diabetes occur in families in which another first-degree relative has type 1 diabetes ***(Petrovsky and Schatz, 2003).*** Point estimates of risk of concordance in monozygotic twin range from 30% to 70%. Approximately 50% of the genetic risk for type 1 diabetes is attributed to HLA region. HLA located on short arm of chromosome 6 is responsible for genetic susceptibility to type 1 diabetes ***(Sperling, 2004).*** In addition, a minimum of 11 other loci on different chromosomes has been associated with increased risk of the development of diabetes. Moreover, the insulin gene on chromosome 11 may account for about 10% of the genetic risk ***(Atkinson and Eisenbarth, 2001).***

***B) Environmental factors*:**

Environmental agents might function as initiating factors for diabetes or might act as precipitating factors that convert preclinical diabetes into clinical disease in genetically susceptible individuals. An environmental trigger probably initiates autoimmunity. This results in β-cell injury, impairment of β-cell function, and reduction of β-cell mass ***(Jun and Yoon, 2004).***

These environmental factors include:

1) Viruses:

Newly diagnosed cases appear with greater frequency during autumn and winter ***(ISPAD, 2014).*** These seasonal variation, have been considered, as indirect evidence for infections exposure as congenital rubella, mumps, coxsackie B4, entero virus and others. These viruses, are known, to induce pancreatitis especially the coxasackie viruses, and it has been shown that these viruses can infect human beta cells in vitro.

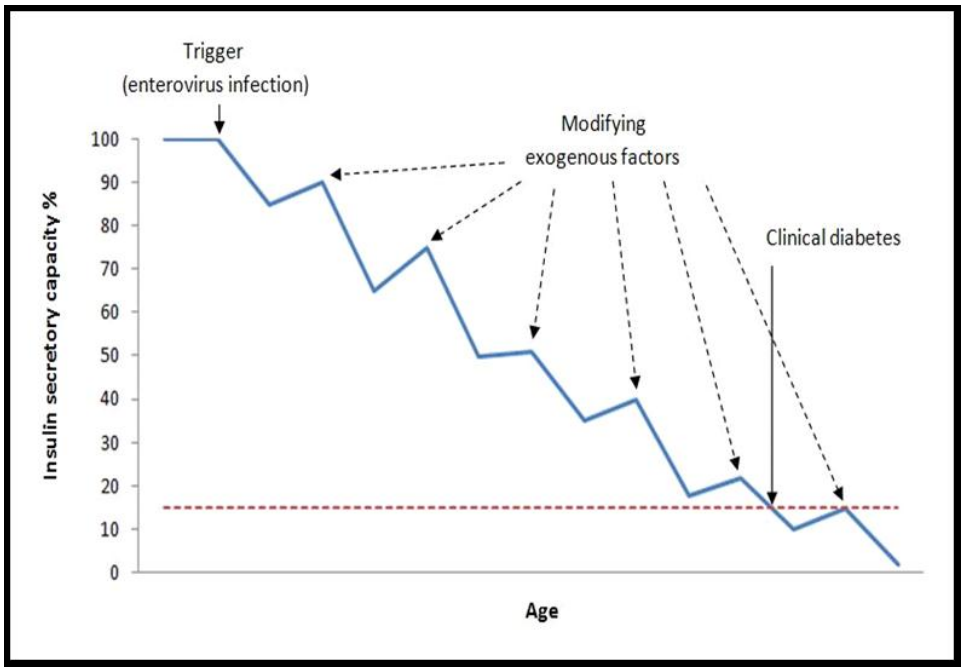

Figure (2): Progression from genetic susceptibility to overt type 1 diabetes. Apparently, the disease process, is triggered by, an exogenous factor, driven by other environmental determinant, and modified by a series of environmental factors in individuals with increased genetic disease susceptibility ***(Knip et al., 2010).***

2) Diet:

The role of cow's milk in the genesis of diabetes is controversial ***(Kimpimaki et al., 2002)*** suggested that about term breast feeding and the early introduction of cow's milk-based infant formula predispose young children who are genetically susceptible to type 1 DM to progressive signs of β-cell autoimmunity.

The likely mechanism is the molecular mimicry between a 17 amino acid peptide of bovine serum albumin and the islet cell antigen 69 ***(Virtanen et al., 2003)***.

3) Role of auto-Immunity:

Chronic T-cell mediated autoimmune destruction of the insulin-secreting ß-cells is the most common cause of type 1 diabetes ***(Lindly et al., 2005).*** The histological hallmark is insulinitis, mononuclear infiltration of pancreatic islets leading to specific destruction of pancreatic beta cells leading to loss of insulin production ***(Petrovsky and Schatz, 2003).***

Patients with immune-mediated type 1 diabetes, are more frequently, affected by autoimmune disorders such as thyroid or adrenal disease, vitiligo, or pernicious anemia ***(Pillai et al., 2011).***

Cytoplasmic islet cell antibodies (ICA) are present in about 80% of children at diagnosis ***(Lindly et al., 2005).***

It was demonstrated that, the Glutamic Acid Decarboxylase (GAD) antibodies have been shown to appear several years before the onset of the disease and in some cases before the detection of islet cell antibodies (ICA). Moreover (GAD) tend to persist compared to (ICA). GAD antibodies known to have inhibitory effect on islet insulin secretion ***(Sperling, 2004).***

Table (2): Criteria for the diagnosis of diabetes mellitus ***(International Society of Pediatric and Adolescent Diabetes ISPAD, 2014)***

- Classic symptoms of diabetes or hyperglycemic crisis, with plasma glucose concentration ≥ 11.1 mmol/L (200 mg/dL)

OR

- Fasting plasma glucose ≥ 7.0 mmol/L (≥ 126 mg/dL). Fasting is defined as no caloric intake for at least 8 h*

OR

- Two hour postload glucose ≥ 11.1 mmol/L (≥ 200 mg/dL) during an OGTT*.

The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water or 1.75 g/kg of body weight to a maximum of 75 g.

OR

- HbA1c > 6.5% †

The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.

HbA1c, hemoglobin A1c; OGTT, oral glucose tolerance test

*****In the absence of unequivocal hyperglycemia, the diagnosis of diabetes based on these criteria should be confirmed by repeat testing.

†A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests. The role of HbA1c alone in diagnosing type 1 diabetes in children is unclear.