INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by T-cell mediated destruction of the pancreatic β-cells, resulting in insulin deficiency and elevated blood glucose levels ***(Daneman, 2006)***.

The increasing incidence of type 1 diabetes in many countries challenges health systems because the disease is presently incurable with no known method of prevention ***(James et al., 2014).*** Around 490,100 children live with the disease worldwide, with incidence estimated to be increasing in children under 15 years by 2.8% per year ***(Catanzariti et al., 2009).*** This trend is particularly worrying because type 1 diabetes increases mortality and morbidity population-wide ***(James et al., 2014).***

Vascular co-morbid diseases include retinopathy, which may cause reduced vision and blindness, and nephropathy, which may result in renal failure and require dialysis or kidney transplantation. This is in addition to hypertension, which is linked to peripheral, cardio- and cerebrovascular disease, the end points of which are limb amputations, cardiac failure, stroke and sudden death. As vascular complications curtail both life expectancy and quality of life ***(Marshall and Flyvbjerg, 2009)*** development at younger ages when people are typically establishing careers and families is particularly detrimental ***(James et al., 2014)***.

Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1), the main oxidized low-density lipoprotein (OxLDL) in endothelial cells is involved in several cellular processes. Oxidized LDL (OxLDL) acts through the interaction with several scavenger receptors, expressed differentially on the surface of the cells of the arterial wall and inflammatory circulating cells ***(Pirillo and Catapano, 2013)***. Among these receptors, LOX-1 has been identified as the main endothelial receptor for OxLDL; however, also macrophages and smooth muscle cells (SMCs) express LOX-1. Several proinflammatory stimuli, including tumor necrosis factor α (TNFα), C-reactive protein (CRP), interleukin-1 (IL-1), angiotensin II, and endothelin-1, and proatherogenic conditions (dyslipidemia, diabetes, hypertension) increase LOX-1 expression ***(Xu et al., 2013)***. Through the interaction with LOX-1, OxLDL activates endothelial cells and induces endothelial dysfunction, smooth muscle cells proliferation, and apoptosis; participates in the transformation of macrophages into foam cells, and induces platelet activation. LOX-1 is undetectable in healthy vessels but overexpressed under pathological conditions ***(Pirillo and Catapano, 2013)***.

The LOX-1 expressed on the cell surface can be proteolytically cleaved and released in a soluble form (sLOX-1) in the circulation under pathological conditions. As elevated plasma levels of soluble receptors may reflect the increased expression of membrane-bound receptors and disease activities,

circulating sLOX-1 has been suggested as a potential cardiovascular disease biomarker ***(Pirillo and Catapano, 2013)***.

Peripheral artery disease (PAD) is characterized by an atherosclerotic occlusive disease of the lower extremities, and diabetes and smoking are the strongest risk factors for PAD. In type 2 diabetic patients, serum sLOX-1 levels were significantly higher in subjects with PAD than in those without ***(Fukui et al., 2013)***. However, levels of sLOX-1 have not been explored in type 1 diabetes.

Aim of the Work

The aim of this study is to determine soluble Lectin-Like Oxidized Low Density Lipoprotein Receptor-1 (sLOX-1) levels in children and adolescents with type 1 diabetes mellitus as a potential marker for diabetic vascular complications and assess its relation to the clinicopathological characteristics of patients, glycemic control and carotid intima media thickness as an index for subclinical atherosclerosis.

Chapter 1
TYPE 1 DIABETES MELLITUS

Definition and description

The term diabetes mellitus describes a complex metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Inadequate insulin secretion and/or diminished tissue responses to insulin in the complex pathways of hormone action result in deficient insulin action on target tissues, which leads to abnormalities of carbohydrate, fat, and protein metabolism. Impaired insulin secretion and/or action may coexist in the same patient ***(ADA, 2014).***

While the etiology of diabetes is heterogeneous, most cases of diabetes can be classified into two broad etiopathogenetic categories (discussed in further detail below): type 1 diabetes, which is characterized by an absolute deficiency of insulin secretion; or type 2 diabetes, which results from a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. While type 1 diabetes remains the most common form of diabetes in young people in many populations, especially those of Caucasian background, type 2 diabetes has become an increasingly important public health concern globally, see the ISPAD guideline on type 2 diabetes ***(Zeitler et al., 2014).***

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).***

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 ***(Wtkins 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.

The possibility of other types of 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.

Epidemiology of type 1 diabetes

Age:

Type 1 diabetes accounts for over 90% of childhood and adolescent diabetes, while across the lifespan, type 1 diabetes accounts for 5–10% of individuals with diabetes. Overall, approximately 80 000 children under 15 yr are estimated to develop type 1 diabetes annually worldwide.Older epidemiological incidence studies define the 'onset of type 1 diabetes' by the date of the first insulin injection because of the variable time between the onset of symptoms and diagnosis, while current guidelines define diabetes based on abnormal test results ***(IDF, 2013).***

Peaks of presentation occur in two age groups:

- ***First peak:*** 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, a fact that distinguishes T1DM from most autoimmune illnesses, which tend to affect females more frequently ***(Justin et al., 2013).***

Gender differences in the incidence of type 1 diabetes are found in some, but not all, populations. However, a male gender bias is generally observed in older adolescents and young adults ***(Wandell and Carlsson 2013).***

There is no apparent correlation with socioeconomic status ***(Alemzadeh and Wyatt, 2008).***

Seasonal variations:

In most studies, a seasonal variation in onset has been observed in children, with the highest incidence of T1DM occurring during the winter months and the lowest occurring during the summer months. This finding may result from winter months having higher rates of viral infections, which cause a metabolic stress that exceeds the ability of the residual β-cells mass to produce insulin sufficient to maintain euglycemia. Some other reports demonstrate higher rates in warmer seasons ***(Skordis et al., 2012).*** In addition, development of islet autoimmunity also demonstrates seasonal variation, as does the association between month of birth and risk of type 1 diabetes ***(Khan et al., 2009).***

Place:

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

A rise in type 1 diabetes incidence has been observed globally in recent decades which is associated with an increased proportion of individuals with low-risk HLA genotypes in some populations, suggesting an increasing role for environmental factors in the disease etiology. In some reports there has been a disproportionately greater increase in those under the age of 5 yr and in developing countries or those undergoing economic transition in recent decades ***(Sipetic et al., 2013).*** There is evidence for a plateau in incidence in some countries in recent years 19 ***(Skrivarhaug et al., 2014).***

Epidemiology of type1 diabetes in Egypt

A study of the incidence and prevalence of Type1 diabetes was held in four Egyptian Governates (Fayoum, Minofya, Sinai, Suez) showed a prevalence rate of 0.7/1000 and incidence rate of 4/100.000 ***(Salem et al., 2007).***

Etiology and Pathogenesis of type 1 diabetes:

The etiology is multifactorial, however, the specific roles for genetic susceptibility, environmental factors, the immune system, and β-cells in the pathogenic processes underlying type 1 diabetes remain unclear.

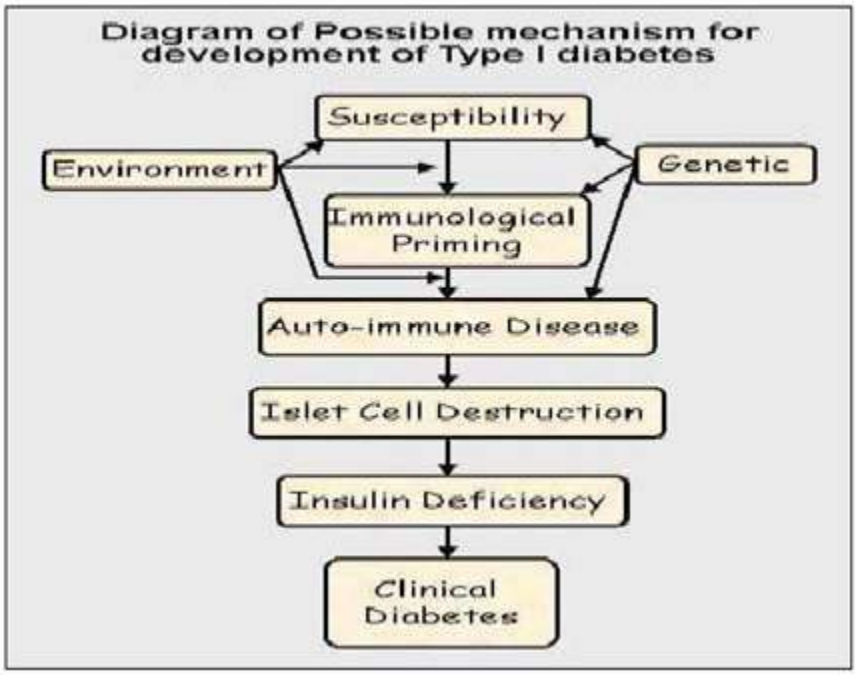

Figure (1): Possible mechanism for development of T1DM ***(Lamb, 2011)***

A) Genetic predisposition:

Genetic predisposition account for about one third of susceptibility to type 1 diabetes ***(Bain et al., 2003)***. It has been recognized that susceptibility to type 1 diabetes is partly inherited ***(Herr et al., 2000).***

Familial aggregation accounts for approximately 10% of cases of type 1 diabetes, but more than 20% when accounting for the extended family history ***(Parkkola et al., 2012),*** however, there is no recognizable pattern of inheritance. The risk of diabetes to an identical twin of a patient with type 1 diabetes is <40% ***(Knip, 2011);*** for a sibling the risk is

approximately 4% by age 20 yr and 9.6% by age 60 yr ***(Zhao et al., 2014);*** compared with 0.5% for the general population. The cumulative risk of diabetes by age 15 is greater in HLA-identical DR3-DQ2/DR4- DQ8 siblings (17 vs. 6% in those sharing one haplotype or none). The risk is also higher in siblings of probands diagnosed at younger age, paternal young onset diabetes, male sex, and older parental age ***(Gillespie et al., 2014).***

Type 1 diabetes is two to three times more common in the offspring of diabetic men (3.6–8.5%) compared with diabetic women (1.3–3.6%).The cumulative risk of type 1 diabetes is approximately 4% for offspring of adult onset (15–39 yr) type 1 diabetes, with a similar recurrence risk in the offspring of mothers and fathers ***(Harjutsalo et al., 2010).***

Susceptibility to autoimmune type 1 diabetes is determined by multiple genes; with more than 60 risk loci identified by genome-wide association studies. Human leukocyte antigen (HLA) genotype confers approximately 50% of risk ***(Howson et al., 2011).***

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).***

HLA antigens are glycoprotein found on the surface of cells. They comprise classes I and II, which are encoded by different genes within the HLA region and this differ fundamentally in their structure.; in the Caucasian population, specific combinations of HLA DR and DQ alleles determine genetic susceptibility ***(Nguyen et al., 2013).*** The highest-risk haplotypes are DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04-DQA1*03:01-DQB1*03:02 (also expressed as DR3/DR4 or DQ2/DQ8 using the former serological designation). Haplotypes conferring protection from type 1 diabetes are DRB1*15:01-DQA1*01:02-DQB1*06:02, DRB1* 14:01-DQA1*01:01-DQB*05:03, and DRB1*07:01- DQA1* 02:01-DQB1*03:03. For individuals who are heterozygotes for the two highest risk HLA haplotypes (DR3/4), the odds ratio is 30 for development of islet autoimmunity and type 1 diabetes ***(Erlich et al., 2008),*** however, <10% of those with HLA conferred diabetes susceptibility genes progress to clinical disease ***(Knip, 2011).***

B) Role of auto-Immunity:

Type 1 diabetes is characterized by chronic immunemediated destruction of pancreatic β cells, leading to partial, or in most cases, absolute insulin deficiency. The majority of cases (type 1A) result from autoimmune mediated

pancreatic β-cell destruction, which occurs at a variable rate, and becomes clinically symptomatic when approximately 90% of pancreatic β cells are destroyed.

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 ***(Pilia et al., 2011).***

Diabetes-associated autoantibodies, which are serological markers of β-cell autoimmunity, include GAD, ICA, IA2, IAA, and ZnT8 ***(Watkins et al., 2014).*** The expression of these antibodies is age-dependent, with IAA and ZnT8 more commonly expressed in children aged <10 yr, while GAD and IA-2 are associated with older age and GAD with female gender ***(Howson 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).***

When the clinical presentation is typical of type 1 diabetes but antibodies are absent, then the diabetes is classified as type 1B (idiopathic). Most cases are of African or Asian ancestry, however, other forms of diabetes, including type 2 and monogenic diabetes, should also be considered**.** Individuals at increased risk of developing type 1 diabetes can be identified by a combination of diabetes-associated autoantibodies, genetic markers, intravenous glucose tolerance test (IVGTT) and/or OGTT ***(Bonifacio et al., 2014).***

C) 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 an β-cell injury, impairment of β-cell function, and reduction of β-cell mass ***(Jun and Yoon, 2004).*** The process usually begins months to years before the manifestation of clinical symptoms ***(Ziegler et al., 2013).***