INTRODUCTION

Diabetes is a chronic illness that requires continuing medical care and patient self-management education to prevent acute complications and to reduce the risk of long-term complications. Diabetes care is complex and requires that many issues, beyond glycemic control, be addressed. A large body of evidence exists that supports a range of interventions to improve diabetes outcomes ***(Tasali and Ip, 2008)***.

Type 1 diabetes is associated with a high risk for early atherosclerotic complications. Patients have a 4-fold (in men) to 8-fold (in women) excess risk of coronary heart disease compared with that for the general population ***(Swerdlow and Jones, 1996)***. As in adults with type 2 diabetes, carotid intima media thickness (CIMT) is significantly increased in type 1 diabetes suggesting a precocious development of atherosclerosis in this disease ***(Nathan et al., 2003)***. Increased intimal thickness is associated with peripheral endothelial dysfunction, age of onset of diabetes, insulin dose requirement, systolic blood pressure and total cholesterol levels ***(Vigili de Kreutzenberg et al., 2009)***.

Plasminogen activator inhibitor 1 (PAI-1) is a fast-acting inhibitor of fibrinolysis that alters the thrombotic-fibrinolytic equilibrium in favor of occlusion ***(Hamsten et al., 1987)***. High PAI-1 levels are associated with an increased cardiovascular risk of atherothrombosis. Furthermore, increased plasma PAI-1

levels are associated with dyslipidemia, hyperinsulinemia and hypertension ***(Bastard et al., 2000; Cesari et al., 2010)***.

Although PAI-1 is powerfully associated with hyperinsulinemia and insulin resistance in population studies, the mechanism of such an association is potentially complex. Thus, a number of factors associated with the insulin resistance cluster are able to induce expression or secretion of PAI-1 by isolated liver cell or endothelial cell lines in tissue culture, including triglyceride-rich lipoproteins, oxidized low density lipoprotein (LDL), proinsulin-like molecules, and insulin itself ***(Yudkin, 1999)***.

In obesity and diabetes, PAI-1 has been linked to the increased incidence of thrombosis. It plays a critical role in the insulin resistance syndrome, which leads to type 2 diabetes mellitus, and is associated with its side effects such as an increased risk of diabetic nephropathy, atherosclerotic cardiovascular disease and others ***(Jankun et al., 2012)***.

AIM OF THE WORK

The aim of this study was to:

1) Determine serum levels of PAI-1 in children and adolescents with type 1 diabetes and its relation to metabolic control.
2) Assess the relation between PAI-1 levels and CIMT as synergistic risk factors for development of atherosclerosis.

Chapter 1

DIABETES MELLITUS

Definition:

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels ***(American Diabetes Association, 2012)***.

Type 1 diabetes mellitus (T1DM) is a heterogeneous disorder characterized by autoimmune-mediated destruction of pancreatic beta cells that culminates in absolute insulin deficiency. T1DM is most commonly diagnosed in children and adolescents, usually presents with symptomatic hyperglycemia, and imparts the immediate need for exogenous insulin replacement ***(Haller et al., 2005)***. It seems that two peaks of T1DM presentation occur in childhood, one between 5 and 7 years of age and the other at puberty ***(Haller et al., 2005)***.

Diabetes mellitus, commonly referred to as diabetes was first identified as a disease associated with "*sweet urine*," and excessive muscle loss in ancient world. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine ***(Conrad, 2012)***.

Type 1 diabetes is an important risk factor for cardiovascular events. Individuals with diabetes have 2-fold to 4-fold increased risk of developing atherosclerotic diseases, observations from postmortem studies have indicated that atherosclerosis in young adults is associated with the prediabetic state. Therefore, patients with type 1 diabetes mellitus in childhood may be at high risk of developing subsequent cardiovascular disease ***(Järvisalo et al., 2004)***.

Atherosclerosis is the major cause of the morbidity and mortality in type 1 diabetes mellitus. Carotid intima media thickness (CIMT) is the early sign of atherosclerosis and thereby, also the sign of macrovascular diseases ***(Gül et al., 2010)***.

Epidemiology:

The incidence of type 1 diabetes varies by geography with 1 in 300 affected worldwide ***(Vehic et al., 2011)***. Differences in disease prevalence and changes in incidence rates suggest that a combination of multiple genetic and environmental factors contribute to T1DM risk ***(Haller et al., 2005)***. The estimated prevalence of T1DM in children and adolescents is 0.38/1000 in Egypt ***(Salem et al., 2010)***.

In the western hemisphere, DM is one of the most prevalent chronic diseases in childhood, whereas the incidence of T1DM in developing countries is significantly less than that in the western hemisphere. Epidemiological studies indicate

that there is gradual but steady increase in the incidence of both T1DM and T2DM in both developed and developing countries ***(Dejkhamron et al., 2007)***.

Over the past 30 years, the ability to predict the development of T1DM has improved dramatically with the combined use of genetic, autoantibody, and metabolic markers ***(Haller et al., 2005)***.

The rising incidence and decreasing age at diagnosis of type 1diabetes is accounted for by the impact of environment on children with lower-risk HLA class II genes, who previously would not have developed type 1 diabetes in childhood ***(Fourlanos et al., 2008)***.

Because clinical T1DM typically does not present until approximately 80% to 90% of the beta cells have been destroyed, there is a marked gap between the onset of autoimmunity and the onset of diabetes ***(Haller et al., 2005)***.

a) Age:

Diabetes is one of the most common diseases in school-aged children. According to the 2011 National Diabetes Fact Sheet, about 215,000 young people in the US under age 20 had diabetes in 2010. This represents 0.26 percent of all people in this age group ***(National Diabetes Fact sheet, 2011)***.

Peaks of presentation occur in 2 age groups: at 5-7 years of age and at the time of puberty. The first peak may

correspond to the time of increased exposure to infectious agents coincident with the beginning of school; the second peak may correspond to the pubertal growth spurt induced by gonadal steroids and the increased pubertal growth hormone secretion (which antagonizes insulin) ***(Alemzadeh and Ali, 2011)***.

On average, in children under age 15, type 1 diabetes incidence increases as a child gets older. In other words, a person 10-14 years old has a higher risk of developing type 1 diabetes, someone 5-9 years old has a middle risk, and someone 0-4 years old has a lower risk. Someone 10-14 has about twice the risk of developing type 1diabetes as someone under 5. This trend generally does not vary by gender ***(Diamond Project group, 2006)***.

There is also a relatively high incidence in people in their late 30s and early 40s, in whom the disease tends to present less aggressively (with early hyperglycemia without ketoacidosis and gradual onset of ketosis). This slower-onset adult form of T1DM is referred to as latent autoimmune diabetes of the adult (LADA) ***(National diabetes fact sheet, 2011)***.

b) Season:

The incidence of T1DM varies with seasonal changes and geography. Incidence rates are higher in autumn and winter and are lower in the summer ***(Haller et al., 2005)***.

c) Race:

Type 1 diabetes is more common among non-Hispanic whites, followed by African Americans and Hispanic Americans, it is comparatively uncommon among Asians ***(Lal et al., 2011)***.

d) Countries:

Internationally, rates of type 1 diabetes are increasing. In Europe, the Middle East, and Australia, rates of type 1diabetes are increasing by 2-5% per year ***(Imkampe and Gulliford, 2011)***. Scandinavia has the highest prevalence rates for T1DM (i.e. approximately 20% of the total number of people with DM), while China and Japan have the lowest prevalence rates, with less than 1% of all people with diabetes ***(Khardori and Griffing, 2012)***.

Classification:

Classification of diabetes includes four clinical classes ***(ADA, 2012)*** **(Table 1):**

- **Type 1 diabetes** (β-cell destruction, usually leading to absolute insulin deficiency) which further subdivided into:
  - ***Immune- mediated diabetes.***
  - ***Idiopathic diabetes.***
- **Type 2 diabetes** (ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance).

- **Other specific types of diabetes due to** (Genetic defects of the β-cell, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug- or chemical-induced diabetes, infections).
- **Gestational diabetes mellitus** (GDM).

Table (1): Classification of DM

<table>
<tr><td colspan="2">I. Type 1
β -cell destruction, usually leading to absolute insulin deficiency
A. Immune mediated
B. Idiopathic</td></tr>
<tr><td colspan="2">II. Type 2
May range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance</td></tr>
<tr><td colspan="2">III. Other specific types</td></tr>
<tr><td>A. Genetic defects of β -cell function
1. Chromosome 12, HNF−1α (MODY3)
2. Chromosome 7, glucokinase (MODY2)
3. Chromosome 20, HNF−4α (MODY1)
4. Chromosome 13, insulin promoter factor- (IPF-1; MODY4)
5. Chromosome 17, HNF−1β (MODY5)
6. Chromosome 2 (MODY6)
7. Mitochondrial DNA mutation
8. Chromosome 7, KCNJ11
9. Others</td><td>E. Drug- or chemical-induced
1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. β-adrenergic agonists
8. Thiazides
9. Dilantin
10. α -Interferon
11. Others</td></tr>
<tr><td>B. Genetic defects in insulin action
1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipoatrophic diabetes
5. Others</td><td>F. Infections
1. Congenital rubella
2. Cytomegalovirus
3. Others</td></tr>
</table>

C. Diseases of the exocrine pancreas 1. Pancreatitis 2. Trauma / pancreatectomy 3. Neoplasia 4. Cystic fibrosis 5. Haemochromatosis 6. Fibrocalculouspancreatopathy 7. Others	**G. Uncommon forms of immune-mediated diabetes** 1. ‘‘Stiff-man’’ syndrome 2. Anti-insulin receptor antibodies 3. Others 4. Polyendocrine autoimmune deficiencies APS I and II
D. Endocrinopathies 1. Acromegaly 2. Cushing’s syndrome 3. Glucagonoma 4. Phaeochromocytoma 5. Hyperthyroidism 6. Somatostatinoma 7. Aldosteronoma 8. Others	**H. Other genetic syndromes sometimes associated with diabetes** 1. Down syndrome 2. Klinefelter syndrome 3. Turner syndrome 4. Wolfram syndrome 5. Friedreich’s ataxia 6. Huntington’s chorea 7. Laurence-Moon-Biedl syndrome 8. Myotonic dystrophy 9. Porphyria 10. Prader-Willi syndrome 11. Others
IV. Gestational diabetes	

(*ADA, 2012)*

Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient ***(ADA, 2012)***.

Table (2): Characteristics of type 1 and type 2 diabetes in children and adolescents

Characteristic	Type1	Type2	MODY
Age at onset	Throughout childhood	Pubertal	Pubertal
Race	All (low incidence in Asians)	Native American, African American, Hispanic	Caucasian
Onset	Acute severe; DKA common	Insidious; ketosis less common	Gradual
Obesity	As in population	Common	Uncommon
Acanthosisnigricans	Absent	Present	Absent
Insulin secretion	Decreased/absent	Variable	Variably decreased
Insulin sensitivity	Normal	Decreased	Normal
Insulin dependency	Permanent	Episodic	Infrequent
Pancreatic	70-80% ICA positive	ICA negative	ICA negative
autoantibodies	85-98% GAD positive	GAD positivity ±	GAD negative
Family history	5-15%	75-90%	100%
Ethnicity	All (low incidence in Asians)	Native American, African American, Hispanic	Caucasian
Mode of inheritance	Nonmendelian, sporadic	Nonmendelian, familial	Autosomal dominant

(Dejkhamron et al., 2007)

TYPE 2 DIABETES MELLITUS:

This form of diabetes, which accounts for 90-95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency**.** Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur ***(ADA, 2012)***.

T2DM is considered a polygenic disease aggravated by environmental factors, such as low physical activity and excessive caloric intake. Most patients are obese, though the disease can occasionally be seen in normal weight individuals ***(Alemzadeh and Ali, 2011)***.

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region ***(ADA, 2012)***. The genetic basis for T2DM is complex and incompletely defined; no single identified defect predominates as does the human leucocyte antigen (HLA) association with T1DM ***(Alemzadeh and Ali, 2011)***.

TYPE 1 DIABETES MELLITUS:

Pathogenesis:

T1DM results from the interaction of genetic and environmental factors that alter the immune system and culminate in destruction of the pancreatic β-cell ***(Dejkhamron et al., 2007)***.

- **Genetics:**

Over the last decade, whole genome screens have indicated that there are at least 15 other loci associated with type 1 diabetes, and of those, another 2 genes intimately

associated with T-cell activation have been identified recently ***(Gillespie, 2006)***.

Serological markers of an autoimmune pathologic process, including islet cell or insulin autoantibodies, are present in 85-90% of individuals when fasting hyperglycemia is detected ***(ISPAD, 2009a)***. Individuals at increased risk of developing type 1diabetes can often be identified by measurement of diabetes associated autoantibodies, genetic markers and intravenous glucose tolerance testing ***(ISPAD, 2009a)***.

Susceptibility to autoimmune type 1 diabetes is determined by multiple genes; in a recent meta-analysis more than 40 distinct genomic locations provided evidence for association with T1DM ***(ISPAD, 2009a)***. HLA genes having the strongest known association, there is linkage to specific combinations of alleles with both susceptible or protective haplotypes ***(Erlich et al., 2008)***.

The major type 1 diabetes susceptibility locus maps to the HLA class II genes and accounts for 30-50% of genetic type 1 diabetes risk ***(Steck et al., 2011)***. The first T1DM susceptibility locus identified, the HLA complex, provides the greatest contribution (40%-60%) to genetic susceptibility. There are three classes of HLA genes, with class II genes having the strongest association with T1DM ***(Haller et al., 2005)***.

Children of mothers who have T1DM have only a 2% risk of developing T1DM, whereas children of fathers who have T1DM have a 7% risk ***(Hamalainen and Knip, 2002)*** (Table 3).

Table (3): Genetic susceptibility to type 1 diabetes mellitus

General population: 0.3%
Relatives: 2–50%
Twins 1. Monozygotic: 30%–50% 2.Dizygotic: 6%–10%
Siblings: 5%
Offspring 1. Of affected father: 7% 2. Of affected mother: 2%
Parents: 3%

(Hamalainen and Knip, 2002)

- **<u>Environment</u>:**

Environmental factors such as virus, dietary factors, and pollutants are implicated in disease pathogenesis ***(Lammi et al., 2005)***.

a) Viruses:

Several viruses have been identified to be associated with the development of T1DM with the strongest evidence linking the rubella virus. Individuals with congenital rubella have 20

percent likelihood to develop T1DM in later life ***(Lammi et al., 2005)***. Infection with enterovirus, cytomegalovirus, coxsackie virus, parvovirus B19, and rotavirus in susceptible individuals have also been implicated to play a role in the causation of T1DM ***(Roivainen, 2006)***.

The mechanism(s) of virus-induced β-cell destruction is not fully understood. In certain instances the virus can infect the β-cell and cause a direct cytolysis. A virus also can induce an immune response by molecular mimicry, a mechanism that is implicated with coxsackie virus B4 that shares sequence homology with the β-cell antigen, glutamic acid decarboxylase (GAD65) ***(Dejkhamron et al., 2007)***.

b) Diet *(Alemzadeh and Ali, 2011)*:

Breast-feeding may lower the risk of T1DM, either directly or by delaying exposure to cow's milk protein. Early introduction of cow's milk protein and early exposure to gluten have both been implicated in the development of autoimmunity and it has been suggested that this is due to the "leakiness" of the immature gut to protein antigens.

Antigens that have been implicated include β-lactoglobulin, a major lipocalin protein in bovine milk, which is homologous to the human protein glycodelin, a T-cell modulator. Other dietary factors that have been suggested at various times as playing a role in decrease diabetes risk include omega-3 fatty acids, vitamin D, ascorbic acid, zinc, and vitamin E.