Introduction

Diabetes mellitus (DM) is a syndrome characterized by a loss of glucose homeostasis. The disease is progressive and associated with a long list of complications. The prevalence of type 1 DM in childhood is increasing with a worldwide annual increase estimated at 3% (range 2- 5%) ***(Rewers et al., 2004).***

Diabetic nephropathy (DN) is a serious complication of type 1 DM affecting approximately one third of patients. The principle pathogenic factor of DN is microangiopathy, representing functional and structural abnormalities in the microvascular system ***(Chiarelli et al., 2000)***. Chronic hyperglycemia is central in the pathophysiology of microangiopathy and in the evolution of DN ***(Casani et al., 2000)***. It leads to a series of biochemical disturbances in the kidney leading to functional changes and followed by irreversible structural changes presented later on as a clinical disease ***(Larkins and Dunlop, 1992)***.

Clinically - evident diabetes - related renal disease is extremely rare in childhood. However, early functional and structural abnormalities may be present many years before clinical manifestations, making the search for a biochemical or radiological markers as early predictors of these changes a health priority ***(Palliccia et al., 2008)***.

The appearance of pathological levels of urinary albumin excretion represents the most common clinical sign of early

renal involvement in patients affected by diabetes mellitus ***(Thomas et al., 2005)***

As is well known, pathological albuminuria and proteinuria constitute the consequence of diffuse diabetes induced glomerular damage. However, renal tubulointerstitium also seems to play an equally important role in the genesis of diabetic nephropathy, as the consequence of a persistent exposure to a variety of metabolic and hemodynamic injuring factors associated with sustained diabetic disease ***(Thomas et al., 2005).***

Furthermore, persistent diabetic proteinuria, secondary to the appearance of manifest glomerular lesions, represents another important cause of tubular injury, as the sustained passage of plasmatic proteins within the tubular lumen is harmful to epithelial cells due to progressive intratubular complement cascade activation. This last condition can lead first to tubular inflammation and then to tubulointerstitial fibrosis, which ultimately signals the appearance of an irreversible renal impairment, leading to chronic kidney disease ***(Abbate et al., 2006)***.

In recent years, neutrophil gelatinase- associated lipocalin (NGAL) has emerged in clinical and experimental nephrology as one of the most promising tubular biomarkers in the diagnostic field of acute and chronic renal diseases. In patients undergoing treatments potentially detrimental to the kidney, such as contrast medium administration and cardiac surgery ***(Hirsch et al., 2007),*** as well as in subjects with

unstable nephropathies ***(Trachtman et al., 2006)***. The increase in NGAL levels predicts the onset of acute kidney injury in the short term, notably anticipating the following increase in serum creatinine levels and thus allowing for the arrangement of preventive therapeutic measures in a timely manner ***(Mishara et al., 2006).***

The Doppler resistance index (RI) (peak systolic velocity — peak end diastolic velocity)/peak systolic velocity) reflects the intrarenal vascular resistance and is widely used for quantifying the alteration in renal blood flow that may occur with renal disease. Elevated RI was reported with vascular interstitial disease, including diabetic nephropathy ***(Palliccia et al., 2008).***

Increased intrarenal resistance index has been shown in adults with DN as a useful marker of potential impairment of renal function in diabetic kidney disease ***(Fruchiger et al., 2000).***

Nonetheless, only a few reports in the literature address this issue in children, and data regarding the relationship between increased intrarenal RI and early clinical stages of DN in children and adolescents with diabetes are scant and controversial.

Aim of the Work

With the present study, we aimed at evaluating the levels of urinary neutrophil gelatinase-associated lipocalin (NGAL), in urine as an early detector of diabetic nephropathy in type 1 DM. and to investigate the possible alteration of intrarenal RI in children with diabetes compared with healthy children.

Diabetes Mellitus

Introduction and Classification:

Definition:

Diabetes is a metabolic disorder of multiple causes characterized by chronic hyperglycemia and disorders of carbohydrate, fat, and protein metabolism. It may be classified as autoimmune mediated type 1 diabetes, or as insulin resistance associated type 2 diabetes, or a combination of these factors. Type 1 diabetes mellitus (T1DM) commonly occurs in childhood or adolescence, although the rising prevalence of type 2 diabetes mellitus (T2DM) in these age groups is now being seen worldwide ***(ADA, 2010)***.

The major forms of diabetes are classified according to those caused by deficiency of insulin secretion due to pancreatic β-cell damage (type 1 DM, or T1DM) and those that are a consequence of insulin resistance occurring at the level of skeletal muscle, liver, and adipose tissue, with various degrees of β-cell impairment (type 2 DM, or T2DM) ***(ADA, 2006)***.

T1DM is the most common endocrine-metabolic disorder of childhood and adolescence, with important consequences for physical and emotional development. Individuals with T1DM confront serious lifestyle alterations that include an absolute daily requirement for exogenous insulin, the need to monitor

their own glucose level, and the need to pay attention to dietary intake ***(Ozmen and Boyuada, 2003)***.

Morbidity and mortality from acute metabolic derangements and from long-term complications (usually in adulthood) that affect small and large vessels resulting in retinopathy, nephropathy, neuropathy, ischemic heart disease, and arterial obstruction with gangrene of the extremities ***(Kuzuya et al.,2002).***

The acute clinical manifestations are due to hypoinsulinemic hyperglycemic ketoacidosis. Autoimmune mechanisms are factors in the genesis of T1DM; the long-term complications are related to metabolic disturbances ***(Balkan and Eschuege, 2003)***.

DM is not a single entity but rather a heterogeneous group of disorders in which there are distinct genetic patterns as well as other etiologic and pathophysiologic mechanisms that lead to impairment of glucose tolerance ***(Gaber et al., 2000)***.

Table (1): Etiologic Classifications of Diabetes Mellitus

Type I diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
Immune mediated, Idiopathic.
Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
Dominant type 2 due to sulfonylurea receptor 1 mutation.
Other specific types
Genetic defects of β-cell function
Genetic defects in insulin action
Diseases of the exocrine pancreas (Pancreatitis, Trauma, pancreatectomy, Neoplasia, Cystic fibrosis, Hemochromatosis, Fibrocalculous pancreatopathy, Pancreatic resection, Others).
Endocrinopathies **(**Acromegaly, Cushing disease, Glucagonoma, Pheochromocytoma, Hyperthyroidism, Somatostatinoma Aldosteronoma, Others.)
Drug-or chemical-induced**(**Vacor, Pentamidine, Nicotinic acid Glucocorticoids, Thyroid hormone**,** Diazoxide, β-Adrenergic agonists, Thiazides, Dilantin, β-Interferon Others**).**
Infections (Congenital rubella, Cytomegalovirus, Others—hemolytic uremic syndrome**).**
Uncommon forms of immune-mediated diabetes**.**
Other genetic syndromes sometimes associated with diabetes**(**Down syndrome, Klinefelter syndrome, Turner syndrome, Wolfram syndrome, Friedreich ataxia, Huntington chorea, Laurence-Moon-Biedl syndrome, Myotonic dystrophy, Porphyria, Prader-Willi syndrome, Others**).**
Gestational diabetes mellitus
Neonatal diabetes mellitus

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

(Balkan and Eschuege, 2003)

Type 1 Diabetes Mellitus (Immune Mediated)

Epidemiology:

Incidence:

The incidence of T1DM is rapidly increasing in specific regions and shows a trend toward earlier age of onset. T1DM accounts for about 10% of all diabetes, affecting 1.4 million in the United States and about 15 million in the world. It is one of the most common severe chronic childhood diseases; 40% of individuals with type 1 DM are younger than 20 yr of age ***(Rewers et al., 2004)***.

Girls and boys are almost equally affected; there is no apparent correlation with socioeconomic status ***(Krischer et al., 2004).***

Peaks of presentation occur in 2 age groups: at 5–7 yr of age and at the time of puberty. A growing number of cases are presenting between 1 and 2 yr of age. The 1st peak may correspond to the time of increased exposure to infectious agents coincident with the beginning of school; the 2nd peak may correspond to the pubertal growth spurt induced by gonadal steroids and the increased pubertal growth hormone secretion (which antagonizes insulin). These possible cause-and-effect relationships remain to be proved ***(Gillespie et al., 2005).***

Most cases (95%) of type 1 diabetes mellitus are the result of environmental factors interacting with a genetically susceptible person. This interaction leads to the development of autoimmune disease directed at the insulin-producing cells of the pancreatic

islets of Langerhans. These cells are progressively destroyed, with insulin deficiency usually developing after the destruction of 90% of islet cells ***(Cruz et al., 2004).***

Etiology and pathogenesis:

A-Genetic issue:

Clear evidence suggests a genetic component in type 1 diabetes mellitus, genes for T1DM may provide susceptibility to, or protection from, the disease. Although many chromosomal loci associated with such activities have been located, few true genes have been identified ***(Barrett et al., 2007)***.

The genetics of T1DM cannot be classified according to a specific model of inheritance. The most important genes are located within the MHC HLA class II region on chromosome 6p21, formally termed (IDDM1), accounting for about 60% genetic susceptibility for the disease. Their specific contribution to the pathogenesis of T1DM remains unclear ***(Bain et al., 2003).***

Monozygotic twins have a 60% lifetime concordance for developing type 1 diabetes mellitus, although only 30% do so within 10 years after the first twin is diagnosed. In contrast, dizygotic twins have only an 8% risk of concordance, which is similar to the risk among other siblings ***(Fernandes et al., 2004).***

The frequency of diabetes developing in children with a diabetic mother is 2-3% and 5-6% if the father has type 1 diabetes mellitus. The risk to children rises to almost 30% if both parents are diabetic ***(Petrovsky and Schatz., 2003)***.

Human leukocyte antigen (HLA) class II molecules DR3 and DR4 are associated strongly with type 1 diabetes mellitus. More than 90% of whites with type 1 diabetes mellitus express one or both of these molecules, compared with 50-60% in the general population ***(Steck et al., 2005).***

Patients expressing DR4 are usually younger at diagnosis and more likely to have positive insulin antibodies, yet they are unlikely to have other autoimmune endocrinopathies, the expression of both DR3 and DR4 carries the greatest risk of type 1 diabetes mellitus; these patients have characteristics of both the DR3 and DR4 groups ***(Lindley et al., 2005)***.

B-Environmental factors:

Factors such as infections and chemicals as well as clues to environmental factors such as seasonality and geographic locations have been suspected of contributing to differences in the incidence and prevalence of T1DM, these agents might function as initiating factors or might act as precipitating factors that convert preclinical diabetes into clinical disease in genetically susceptible individuals. Environmental factors probably initiate autoimmunity. This result in B-cell injury, impairment of B-cell function, and reduction of B-cell mass. ***(Jun and Yoon, 2004).***

Viral infections and vaccinations*:*

Although the etiologic role of viral infections in human T1DM is controversial, Viral infections may be the most important environmental factor in the development of type 1 diabetes mellitus, probably by initiating or modifying an autoimmune process ***(Lammi et al., 2005)***.

Dietary factors:

Dietary factors are also relevant. Breastfed infants have a lower risk for insulin-dependent diabetes mellitus (IDDM). Some cow's milk proteins (e.g., bovine serum albumin) have antigenic similarities to an islet cell antigen ***(Kimpimaki et al., 2001)***. Reduced exposure to UV light and lower vitamin D levels, both of which are more likely found in the higher attitudes, are associated with an increased risk of type 1 diabetes mellitus ***(Mohr et al., 2008)***. Dietary factors have been implicated in the pathogenesis of T1DM, but the role of dietary factors in induction of islet autoimmunity remains controversial.

C- Body Mass Index:

There may be a greater risk of T1DM among individuals who were heavier as young children. The accelerator hypothesis predicts earlier onset in heavier peopl. Insulin resistance is a function of fat mass, and because increasing body weight in the industrialized world has been accompanied by earlier presentation. Therefore, limiting excessive weight gain may be as important for children susceptible to T1DM as for those at genetic risk for T2DM ***(Rosenbloom, 2003)***.

D- Puberty:

The pubertal peak in onset of type 1 DM occurs earlier in girls than boys. This sex difference might be mediated, in part, by estrogen or by genes regulated by estrogen, such as the interleukin-6 (IL6) gene, and suggests that pubertal changes may contribute to accelerated onset of type 1 DM in genetically susceptible females ***(Gillespie et al.,2005)***.

E-Chemical causes:

Drugs such as alloxan, streptozotocin (STZ), pentamidine, and Vacor are directly cytotoxic to β cells and cause diabetes in experimental animals and humans.

Autoimmune Injury:

T1DM is a chronic, T cell–mediated autoimmune disease that results in the destruction of the pancreatic islets. Genetic predisposition and environmental factors lead to initiation of an autoimmune process against the pancreatic islets ***(Lindley et al., 2005).*** It is also assumed that the autoimmune response needs to be sustained against multiple target proteins for prolonged periods of time to overcome protective mechanisms. The autoimmune attack on the pancreatic islets leads to a gradual and progressive destruction of β cells, with loss of insulin secretion. It is estimated that, at the onset of clinical diabetes, 80–90% of the pancreatic islets are destroyed ***(Petrovsky and Schatz, 2003)***. T1DM is associated with other autoimmune diseases such as thyroiditis, celiac disease, multiple sclerosis, and Addison **(*Glastras et al., 2005).***

Pathophysiology:

Insulin performs a critical role in the storage and retrieval of cellular fuel. Its secretion in response to feeding is modulated by the interplay of neural, hormonal, and substrate-related mechanisms to permit controlled disposition of ingested foodstuff as energy for immediate or future use ***(Alemzadeh and Wyatt, 2004).***

Insulin is essential to process carbohydrates, fat, and protein. Insulin reduces blood glucose levels by allowing glucose to enter muscle cells and by stimulating the conversion of glucose to glycogen (glycogenesis) as a carbohydrate store. Insulin also inhibits the release of stored glucose from liver glycogen (glycogenolysis) and slows the breakdown of fat to triglycerides, free fatty acids, and ketones. It also stimulates fat storage. Additionally, insulin inhibits the breakdown of protein and fat for glucose production (gluconeogenesis) in both liver and kidneys ***(Vendrame et al., 2004).***

Hyperglycemia (ie, random blood glucose concentration more than 200 mg/dL or 11 mmol/L) results when insulin deficiency leads to uninhibited gluconeogenesis and prevents the use and storage of circulating glucose. The kidneys cannot reabsorb the excess glucose load, causing glycosuria, osmotic diuresis, thirst, and dehydration. Increased fat and protein breakdown leads to ketone production and weight loss. Without insulin, a child with type 1 diabetes mellitus wastes away and eventually dies due to diabetic ketoacidosis (DKA) ***(Alemzadeh and Wyatt, 2004).***

Lipid metabolism: in uncontrolled type 1 DM there is a rapid mobilization of trigiycerides leading to increased levels of plasma free fatty acids. Mitochondrial oxidation of fatty acids generates acetyl COA which can be further metabolized into ketone bodies, acetoacetate and hydroxybutyrate ***(Gylling et al., 2004).***

These ketone bodies leave the liver and are used for energy production by the brain, heart and skeletal muscles. In type 1 DM, the increased availability of free fatty acids and ketone bodies exacerbate the reduced utilization of glucose furthering the increase in hyperglycemia. Production of ketone bodies in excess of the body ability to utilize them leads to ketoactdosis ***(Alemzadeh and Wyatt, 2004).***

Protein metabolism: insulin deficiency will lead to increased catabolism of protein. The increased rate of proteolysis leads to elevated concentrations in plasma aminoacids. These aminoactds serve as precursors for hepatic and renal gluconeogensis ***(Alemzadeh and Wyatt, 2004).***

Table (2): Criteria for the diagnosis of diabetes mellitus

1. Symptoms of diabetes and a random plasma glucose
>200mg/dl (1 1.1 mmol/I). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
Or 2.Fasting plasma glucose > 126 mg/dl (7.0 mmol/I). Fasting is defined as no caloric intake for at least 8 h.
Or 3.2-h plasma glucose - 200mg/dl (11. 1 mmol/I) during an oral glucose tolerance test. The test should be performed as described by the world health organization, using a glucose load of 75g anhydrous glucose dissolved in water or 1.75 g/kg body wt if weight is < 40 pounds (18kg).

(ADA, 2006)

Presentation and phases of type 1 diabetes:

A: Preclinical diabetes.

B: Presentation of diabetes.

C: Partial remission phase.

D: Chronic phase of life long dependency on administered insulin.

(ISPAD, 2007).

A. Preclinical diabetes:

Preclinical diabetes refers to the months or years preceding the clinical presentation of type 1DM when antibodies can be detected as markers of beta-cell autoimmunity such as ***(ISPAD, 2007):***

1. Islet cell auto antibodies.
2. Gtutamic acid decaroboxylase autoantibodies.
3. IA2 (also known as ICA512or tyrosine phosphatase) auto antibodies.
4. Insulin auto antibodies.

The development of type 1 DM has been divided into a series of stages:

Stage 1: Genetic predisposition.

Stage 2: Triggering of autoimmunity.

Stage 3: Development of a series of auto-antibodies.

Stage 4: Loss of B-cell function, as determined by intravenous glucose tolerance testing (metabolic defects).

Stage 5: Overt DM.

Stage 6: Total or near total B-cell destruction with insulin dependence ***(Petrovsky and Schatz, 2003)***