INTRODUCTION

Diabetes is a group of chronic diseases characterized by hyperglycemia. Modern medical care uses a vast array of lifestyle and pharmaceutical interventions aimed at preventing and controlling hyperglycemia. In addition to ensuring the adequate delivery of glucose to the tissues of the body, treatment of diabetes attempts to decrease the likelihood that the tissues of the body are harmed by hyperglycemia ***(Fong, 2004)*.**

Generally, the injurious effects of hyperglycemia are separated into macro vascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro-vascular complications (diabetic nephropathy, neuropathy, and retinopathy). It is important to understand the relationship between diabetes and vascular disease because the prevalence of diabetes continues to increase, and the clinical armamentarium for primary and secondary prevention of these complications is also expanding ***(Fong, 2004).***

Major long-term complications in patients with diabetes are related to oxidative stress, caused by the hyperglycemia characteristic for diabetes mellitus. These processes are suggested to underlie the pathogenesis for late diabetes complications, including arteriosclerosis and cardiovascular disease. High reactivity of reactive oxygen species (ROS) determines chemical changes in virtually all cellular

components, leading to lipid peroxidation and hence the atherosclerotic changes. The development of CVD is complex, and involves innate immune mediators such as natural killer (NK) cells. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes ***(Brownlee, 2005).***

Natural killer cells are innate lymphocytes involved in the early protection against certain infections and tumors ***(Vidal, 2011).*** Their implication in the pathogenesis of autoimmune disease including Type 1 DM is recognized, although their exact role in this context is complex and still not fully known ***(Rodacki, 2007).*** NKG2D is an activating receptor expressed on cells of the innate and adaptive immune system. It is one of the most intensively studied immune receptors of the past decade. It's unique binding and signaling properties, expression pattern, and functions have been attracting much interest within the field ***(Huntington, 2007*)**.

There are many reports on effects of antioxidants in the management of diabetes. Coenzyme Q10 (CoQ_{10}) has been suggested to decrease CVD risk by largely uncharacterized mechanisms ***(Turunen, 2002)***. As an anti-oxidant, CoQ_{10} has neutralizing effects on ROS, but appears to affect different aspects of the innate immune system independently of its radical scavenging properties e.g. by impacting upon gene expression ***(Schmelzer, 2010)*** and influencing the cytokine response to bacterial stimuli ***(Schmelzer et al., 2009).***

AIM OF THE WORK

The aim of this study is to investigate Natural Killer cells in children and adolescents with type 1 diabetes mellitus; as a component of the innate immune system implicated in the pathogenesis of diabetes and it's long term complications, and the influence of CoQ_{10} supplementation on subset distribution and activation markers of peripheral natural killer cells.

Chapter 1

DIABETES MELLITUS

Definition

The World Health Organization (WHO) defines diabetes mellitus as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both ***(WHO, 1999)***. Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. Ongoing patient self-management education and support are critical to preventing acute complications and reducing the risk of long-term complications ***(ADA, 2015).***

Susceptibility to autoimmune type 1 diabetes is determined by the interaction of multiple genes, with HLA genes having the strongest known association. Progressive beta cell destruction occurs at a variable rate and the disease becomes clinically symptomatic when approximately 90 percent of the pancreatic beta cells are destroyed ***(Kulmala et al., 2000).***

Insulin deficiency then manifests itself clinically as blood glucose levels rise to pathological levels. The onset of the disease is predictable, especially in the relatives of affected

individuals, using a combination of auto-antibody measurements, intravenous glucose tolerance testing and genetic typing ***(Kulmala et al., 2000).***

Historically, infectious agents have been the most frequently noted environmental influences for T1D ***(Boettler, 2011).*** There is, however, no direct evidence that infection plays a role in the pathogenesis of this disease, albeit one agent (i.e., rubella) is often, and incorrectly, cited as evidence for this activity ***(Gale, 2008)***. Beyond rubella infection, a relationship between b-cell autoimmunity and enteroviral infections has long been reported in association with the disease ***(Jaıdane et al., 2010).***

Perhaps second to viruses, nutritional influences have also often been considered in association with T1D, the most predominant being the association between the effect of breastfeeding and/or early exposure to cow's milk on the incidence of autoimmunity and the disorder, albeit here too, the notion remains highly controversial. In support of the notion, a major meta-analysis showed a weak but statistically significant association (OR 1.5) between T1D and both shortened period of breastfeeding and cow's milk exposure before 3–4 mo of age ***(Gerstein, 1994).***

Non-autoimmune type 1 diabetes has similar clinical features but is characterised by the lack of auto-antibodies against the antigens in the islets of Langerhans (islet cell

antibodies- ICA), or beta cell antigens (anti- insulin, anti-GAD65 or anti-IA2 antibodies) ***(WHO, 1999).***

The ability to understand the natural history of T1D has improved dramatically through the combined use of genetic, autoantibody, and metabolic markers of the disease ***(Atkinson, 2005).*** This model for the natural history of T1D suggests that genetically susceptible individuals with a fixed number of b cells are exposed to a putative environmental trigger, which induces b-cell autoimmunity.

This process, marked by the development of islet reactive autoantibodies, portends the development of activated autoreactive T cells capable of destroying b cells, resulting in a progressive and predicable loss in insulin secretory function. With this model, clinical (i.e., symptomatic) T1D does not present until 80%– 90% of the b cells have been destroyed, and there is a marked gap between the onset of autoimmunity and the onset of diabetes ***(Atkinson, 2005).***

However, recently, some aspects of the classical model have been modified to update knowledge gains (Fig. 1) ***(Atkinson, 2001).*** For example, there are data to suggest that pancreatic b cells may persist in some individuals with T1D for an extended period of time (i.e., never reaching zero in many established T1D patients) ***(Meier et al., 2005).*** In addition, the degree of b cell destruction required for symptomatic onset is also of growing question, with recent studies suggesting that

40%–50% b-cell viability may be present at the onset of hyperglycemia ***(Akirav et al., 2008),*** an aspect that may be related to subject age, among other factors (e.g., bodymass index, physical activity, etc.) ***(Matveyenko, 2008).***

This may explain why, despite persistent autoimmunity, insulin secretory function can remain stable for long periods of time in persons with T1D. That said, a loss of first-phase insulin response is usually followed by a period of glucose intolerance and a period of clinically "silent" diabetes ***(Sosenko et al., 2010).*** Finally, the "slope" reflective of b cell loss in the pre-diabetic period has also recently been subject to considerable debate, with some proposing that the disorder may see its symptomatic onset only following period of relapsing/remitting like autoimmunity ***(von Herrath et al., 2007).***

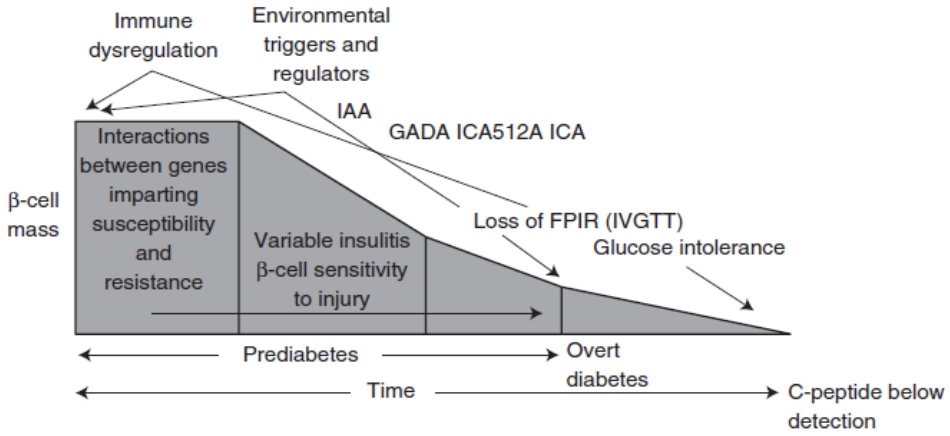

Figure (1): Model of the pathogenesis and natural history of type 1 diabetes. The modern model expands and updates the traditional model by inclusion of information gained through an improved understanding of the roles for genetics, immunology, and environment in the natural history of T1D ***(Adapted from Atkinson and Eisenbarth, 2001).***

Epidemiology: incidence and prevalence

T1D is without question one of the most common chronic diseases of childhood ***(Gale, 2005).*** First, it does appear that two peaks of T1D presentation occur in childhood and adolescence—one between 5 and 7 yr of age, with the other occurring at or near puberty ***(Harjutsalo et al., 2008).*** Beyond this, although many autoimmune disorders disproportionately affect women, T1D appears to affect males and females equally; but controversy does exist whether a modest excess of T1D cases occurs in males in early age or signs of autoimmunity are increased with male gender ***(Krischer et al., 2004).***

In addition, the incidence of T1D varies as a function of seasonal changes, higher in autumn and winter and lower in the summer months ***(Moltchanova et al., 2009).*** The pathogenic mechanisms that underlie these seemingly sure observations are unclear, but, interestingly, recent studies assessing the development of T1D-associated autoimmunity in the months to years before the onset of symptomatic T1D also show a degree of synchronization ***(Kukko et al., 2005),*** thus supporting a theoretical role for an environmental agent driving the pathogenesis of the disorder.

Types

Table (1): Etiological classification of DM: Type 1 Diabetes mellitus (T1DM):

I- Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency). A. Immune mediated. B. Idiopathic.
II- Type 2 diabetes (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: Maturity onset diabetes of the young3 (MODY3), (MODY2), (MODY1), (IPF-1; MODY4), (MODY5) and (MODY6). B. Genetic defects in insulin action: Type A insulin resistance, Leprechauns and Robson-Mendenhall syndrome. C. Disease of the exocrine pancreas: Pancreatitis, Trauma, Pancreatectomy, pancreatic Neoplasia, Cystic fibrosis and Hemochromatosis. D. Endocrinopathy: Acromegaly, Cushing's syndrome, Glucagonoma, Pheochromocytoma, Hyperthyroidism and Somatostatinoma. E. Drug or Chemical Induced: L-asparaginase, glucocorticoids, cyclosporine, tacrolimus and atypical antipsychotics including olanzepine, risperidol. F. Infections: Congenital rubella and Cytomegalovirus. G. Uncommon forms of immune mediated diabetes: "Still-man" syndrome. H. Other genetic syndromes sometimes associated with diabetes: Down Syndrome, Kleinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia and Prader-willy syndrome.
IV. Gestational diabetes mellitus (GDM).

(ISPAD, 2009)

T1DM is a progressive low-insulin catabolic state in which feeding does not reverse but rather exaggerates these catabolic processes ***(Adler et al., 2011).***

Type 1A (The autoimmune form): presence of other autoimmune disorders is highly raised. Susceptibility to type 1A diabetes is determined by multiple genes. In a recent meta-analysis 40 distinct genomic locations provided evidence for association with T1DM ***(Adler et al., 2011).***

2- Type 1B (The idiopathic form): In which there is no evidence of autoimmunity. When the clinical presentation is typical of T1DM, often associated with diabetic ketoacidosis (DKA), but antibodies are absent, then the diabetes is classified as type 1B ***(ISPAD, 2009).***

Type 1c: HbA1c levels were still almost normal at diagnosis, there was a profound lack of insulin, extremely high blood glucose level and full ketoacidosis but there were no anti-islet autoantibody ***(Imagawa et al., 2000).***

Table (2): Clinical and biological characteristics of the different subtypes of type 1 diabetes:

	Type 1a	**Type 1b**	**Type 1c**
Signs of anti-islet autoimmunity	+	-	-
Duration of symptoms before diagnosis	8 months	7 months	< 1 week
Ketosis, Ketoacidosis at diagnosis	Frequent	Frequent	Constant
Blood glucose level at diagnosis	++	++	+++
HBA1c at diagnosis	++	++	Normal or slightly elevated

(Imagawa et al., 2000)

Type 2 Diabetes mellitus (T2DM):

T2DM is characterized by insulin resistance which may be combined with relatively decreased insulin secretion ***(ADA, 2009).*** In the early stage of type 2 diabetes, the predominant abnormality is decreased insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose output by the liver. As the disease progresses, the impairment of insulin secretion occurs and therapeutic replacement of insulin may sometimes be necessary in certain patients ***(McCarthy et al., 2010).***

Pathophysiology

The incidence of type 1 diabetes (T1D) has risen steadily in from the 1950s to the present day, with the recent, alarming prediction that it will double in children under age 5 years by 2020 ***(Patterson et al., 2009).*** We have no known way of preventing this pandemic. There seems little doubt that T1D is a ‘‘disease of civilization,’’ with numerous alterations to our environment since the 1940s and 1950s, including sanitation, healthcare, and diet ***(Cooke, 2009).***

During infancy, the immune system may be provoked with a massive rebuilding of the pancreatic islets and their beta cells via a naturally programmed cell death or apoptosis ***(Ali et al., 2010).*** In children genetically susceptible to T1D with reduced immune tolerance to islet antigens including preproinsulin (PPI), these events could be endogenous initiating factors of disease not requiring exogenous factors.

HLA Molecules and Insulin Peptides:

The categorization of pediatric T1D as an autoimmune disease is not ambiguous, with the presence of the predisposing HLA classII haplotypes, DRB1*04-DQB1*0302 and DRB1*03, in at least 90% of cases and the presence of autoantibodies and, more recently, autoreactive, anti-islet antigen-specific T cells in the circulation of prediabetic individuals and of newly diagnosed and established cases. Transgenic mouse modeling ***(Skowera et al., 2008)*** has provided direct support that it is the peptide-binding activity of

the HLA class II molecules in antigen-presenting cells (APCs) for T lymphocyte peptide recognition that is the main mechanism of action of the DR and DQ molecules in T1D etiology. This is by far the major determinant of disease in the genome. In humans, susceptibility and resistance to T1D has been mapped to particular polymorphic peptide-binding pockets of the DQ molecule, pocket 9, and of DR, pockets 1 and 4. It remains a goal to identify molecules that modulate the function of these pockets and could be therapeutic ***(Zhang et al., 2008).***

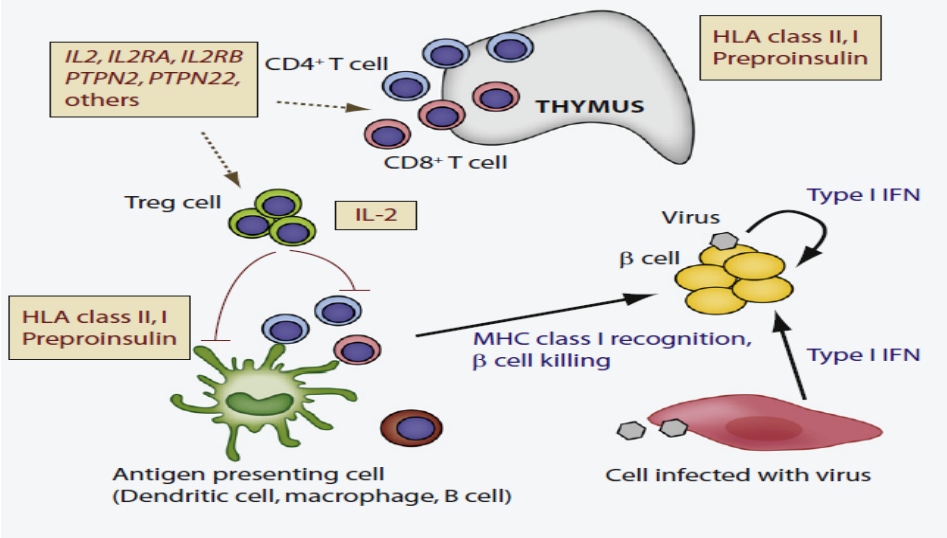

Figure (2): A Model for the Pathogenesis of Type 1 Diabetes Based on Genetic Etiological Studies in Humans Shown are genes (e.g. those that encode MHC HLA class II and class I antigen-presentation molecules, preproinsulin (INS) in the thymus,CTLA-4 in Treg cells, cytokines)believed to be causal in the process of type I diabetes. Also shown are influences of viral infections and type 1 interferon (IFN) production and effects; autoimmune repertoire development in the thymus; and the main immune cell types, CD4+ and CD8+ T cell subsets, T regulatory (Treg) cells, and B cells and various other antigen-presenting cells, acting together to kill pancreatic b cells. Adapted from ***(von Herrath, 2009).***

The identification and description of autoantibodies in T1D have allowed us to gain remarkable insight into the natural history of this disease and, in fact, may comprise the greatest research success story in the 40 years of research investigations into the autoimmune nature of the disease ***(Bonifacio, 2010).*** In combination with a growing understanding of genetic susceptibility, autoantibodies allow for us to accurately predict which patients will develop T1D, from an early age (Fig. 3) ***(Ziegler, 2010).*** Indeed, based on studies of large populations (both general population and families) for metabolic, genetic, and immune markers, levels of risk (i.e., low to very high) can be defined.

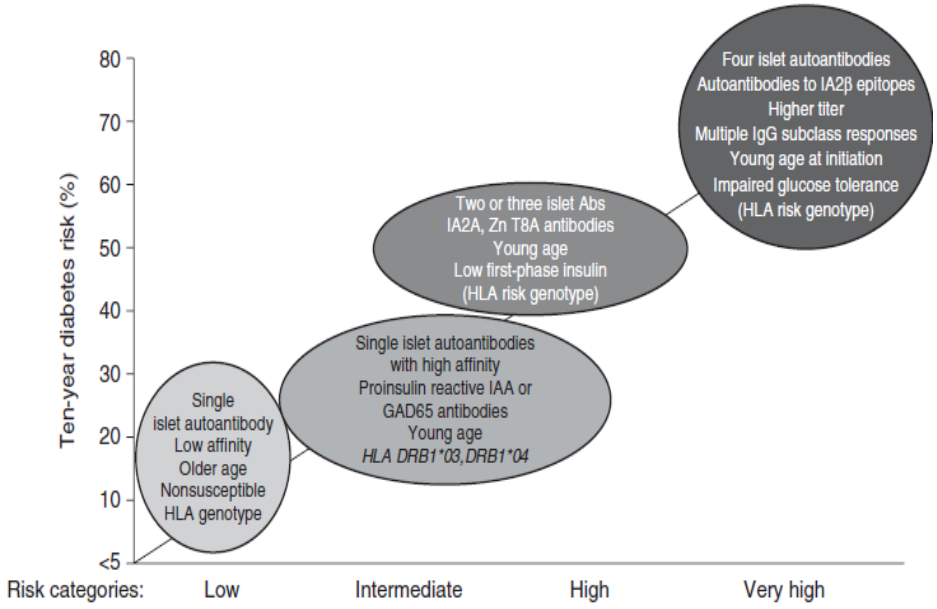

Figure (3): Type 1 diabetes risk stratification by islet autoantibody properties ***(Adapted from Ziegler and Nepom 2010).***

Clinical picture

1. **Preclinical type 1 diabetes** This refers to the months or years preceding the clinical presentation of type 1 diabetes when antibodies can be detected as markers of beta cell autoimmunity: Islet cell autoantibodies (ICA), Glutamic acid decarboxylase autoantibodies (65K GAD isoform),IA2 (also known as ICA 512 or tyrosine phosphatase) autoantibodies and Insulin autoantibodies (IAA) ***(ADA, 2003).***

2. **Presentation of type 1 diabetes** Clinical presentation of diabetes can vary from non-emergency presentations (e.g. polydipsia, polyuria, weight loss, enuresis) to severe dehydration, shock and diabetic ketoacidosis. Prospective follow-up of high-risk subjects shows that diagnosis of type 1 diabetes can be made in asymptomatic individuals in the majority of cases. Some children have a rapid onset of symptoms and present within days in diabetic ketoacidosis; others have a slow onset over several months ***(ADA, 2003).***

3. **Non-emergency presentations:** These include: Recent onset of enuresis in a previously toilet-trained child, which may be misdiagnosed as a urinary tract infection or the result of excessive fluid ingestion. Vaginal candidiasis, especially in prepubertal girls. Chronic weight loss or failure to gain weight in a growing child. Irritability and decreasing school performance. Recurrent skin infections ***(ADA, 2003).***