INTRODUCTION

Defensins are members of small antimicrobial peptides of the innate immune system ***(Chen et al., 2006).*** However, today these peptides are also known as danger signals or "alarmins" playing important roles in inflammation and immunity ***(Oppenheim and Yang, 2005).***

Mammalian defensins are divided into two major families, the α- and β-defensins. Human α-defensins include human neutrophil peptide 1-4 (HNP1-4) and intestinal human defensins (HD-5 and HD-6) produced by Paneth cells. Besides the antimicrobial effects, alpha defensins display chemotactic activity and induce pro-inflammatory cytokines ***(Bowdish et al., 2005).***

HNPs increase the binding of low density lipoprotein (LDL) cholesterol to the endothelial surface suggesting that alpha defensins may modulate the development of atherosclerosis ***(Quinn et al., 2008).***

There is a growing evidence of the role of innate immunity in diabetes. Defensins are members of small antimicrobial peptides of the innate immune system. In addition to their antibacterial and antiviral effects, immunologic functions of defensins has been shown to play a role in the homeostasis ***(Balázs et al., 2014).***

Neutrophil granulocytes are considered to be the primary cellular origin of α-defensins; HNP 1-3 comprise 30%-50% of the granule proteins. HNPs can be released into the extracellular milieu following granulocyte activation as a consequence of degranulation, leakage, cell death, and lysis during inflammation. α-defensins are also involved in the formation of neutrophil extracellular traps ***(Papayannopoulos and Zychlinsky, 2009).***

Human β-defensins make up another family of antimicrobial peptides ***(Pazgier et al., 2006).*** In addition to their antibacterial and antiviral effects, the chemoattractive function of these defensins has been shown to play a role in immunological reactions that protect the host from various pathogens ***(Dürr et al., 2002).***

Autoimmune type 1 diabetes mellitus (T1DM) is the result of defects in immune regulation. However, type 2 diabetes mellitus (T2DM) is also associated with immune defects and inflammation ***(Kalupahana et al., 2012),*** mainly as a consequence of hyperglycaemia-induced oxidative stress due to increased production of reactive oxygen species (ROS).

Decreased serum HBD2 indicates reduced inflammation in T1DM patients. Many studies observed a deficiency of CAMP and HBD1, primarily in T1DM patients. This is in line with a previously reported negative impact of low glucose and insulin levels on the production of beta defensins ***(Barnea et al., 2008).***

Moreover, HβD-2, which is generally induced by proinflammatory stimuli, was decreased after CoQ10 supplementation in T1DM patients. This suggests that diabetes-associated inflammatory processes were reduced by CoQ10. Antimicrobial peptides (AMPs) are genetically encoded and represent a very important part of the innate immune response ***(Zasloff, 2002).***

In mammals, AMPs are present mainly in phagocytic cells of the immune system for the killing of engulfed or invasive bacteria and in epithelial cells for the prevention of pathogen colonization of host tissue ***(Lehrer and Ganz, 2002).*** In addition to a direct antimicrobial effect, AMPs possess the ability to modulate the immune response through a variety of mechanisms ***(Oppenheim and Yang, 2005).*** Although antimicrobial activity is crucial, AMPs also exert immunomodulatory effects, such as chemotaxis, activation of immature dendritic cells, lipopolysaccharide blockage, angiogenesis, and cytokine induction ***(Rivas et al., 2008).***

Over the past 2 decades, several families of AMPs have been described as existing in many living organisms. In humans, there are three families of AMPs, including defensins, cathelicidins, and histatins ***(Harder and Schroder, 2005).*** Nevertheless, there are other antimicrobial and immunomodulatory peptides that are not included within these families, such as granulysin, lactoferrin, and hepcidin ***(Rivas-Santiago et al., 2006).***

AIM OF THE WORK

The aim of this study is to investigate anti-microbial antibody (human beta defensin 2) 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 CoQ10 supplementation on antimicrobial and proinflammatory activity.

Chapter 1
DIABETES MELLITUS

Definition:

Diabetes is a group of metabolic disease characterized by a state of hyperglycemia resulting from defect in insulin secretion or insulin action, or both. The chronic state of hyperglycemia in diabetes is associated with long term damage, dysfunction, and failure of different organs, especially eyes, kidneys, nerves, heart, and blood vessels ***(American Diabetes Association (ADA), 2014).***

Diabetes mellitus has metabolic, vascular and neuropathic components that are interrelated; this makes DM a major health problem with long term microvascular and macrovascular complications. The development and progression of diabetic complications are strongly related to the degree of glycemic control ***(Özmen and Boyuada, 2003).***

Epidemiology:

- In most western countries, 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 15yrs are estimated to develop type 1 diabetes annually worldwide ***(International Diabetes Federation (IDF), 2013).***

- Although type 1diabetes can be diagnosed at any age, it is one of the most common chronic diseases of childhood. Peaks in presentation occur between 5–7 years of age and at or near puberty ***(Harjutsalo et al., 2008).***
- Whereas most autoimmune disorders disproportionately affect women, type 1 diabetes is slightly more common in boys and men ***(Wandell and Carlsson, 2013).***
- The incidence of type 1 diabetes varies with seasonal changes and birth month. More cases are diagnosed in autumn and winter, and being born in the spring is associated with a higher chance of having type 1 diabetes ***(Kahn et al., 2010).***
- Development of type 1 diabetes-associated autoimmunity (ie, formation of islet autoantibodies) in the months or years before onset of symptomatic type 1 diabetes also shows some seasonal synchronization. These concepts support a theoretical role for an environmental agent initiating or driving the pathogenic processes in type 1 diabetes ***(Kukko et al., 2005).***
- Globally, the incidence and prevalence of type 1 diabetes vary substantially. Type 1 diabetes is most common in Finland (>60 cases per 100 000 people each year) and Sardinia (around 40 cases per 100 000 people each year). By contrast, the disorder is uncommon in China, India, and Venezuela (around 0.1 cases per 100 000 people each year) ***(Patterson et al., 2009).***

- The incidence of type 1 diabetes has been increasing worldwide for several decades. In Finland, Germany, and Norway, annual increases in incidence of 2·4%, 2·6%, and 3·3%, respectively, have been reported ***(Ehehalt et al., 2012).***
- In many countries, the rise in incidence of type 1 diabetes has fluctuated, although Sweden has recently seen incidence rates plateau. If incidence rates continue to increase on their existing path, global incidence could double over the next decade ***(Patterson et al., 2009).***
- 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. The incidence varies between 1/100 000 per year (Pakistan) and 8/100 000 per year (Egypt) in children under the age of 15 years ***(El-Ziny et al., 2014).***
- A plethora of environmental influences have been purported to affect the epidemiology of type 1 diabetes, with infant and adolescent diets, vitamin D and vitamin D pathway constituents, and viruses receiving the most focus ***(Blanton et al., 2011; Cooper et al., 2011).***
- Familial aggregation accounts for approximately10% of cases of type 1 diabetes, but more than 20% when accounting for the extended family history, however, there is no recognizable pattern of inheritance ***(Parkkola et al., 2012).***

- The risk of diabetes to an identical twin of a patient with type 1 diabetes is <40% ***(Knip, 2011).***
- The risk for a sibling is approximately 4% by age 20 yr and 9.6% by age 60 yr; compared with 0.5% for the general population ***(Zhao et al., 2014).***
- 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) ***(Gillespie et al., 2014).***
- 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 yrs) type 1 diabetes, with a similar recurrence risk in the offspring of mothers and fathers ***(Harjutsalo et al., 2010).***

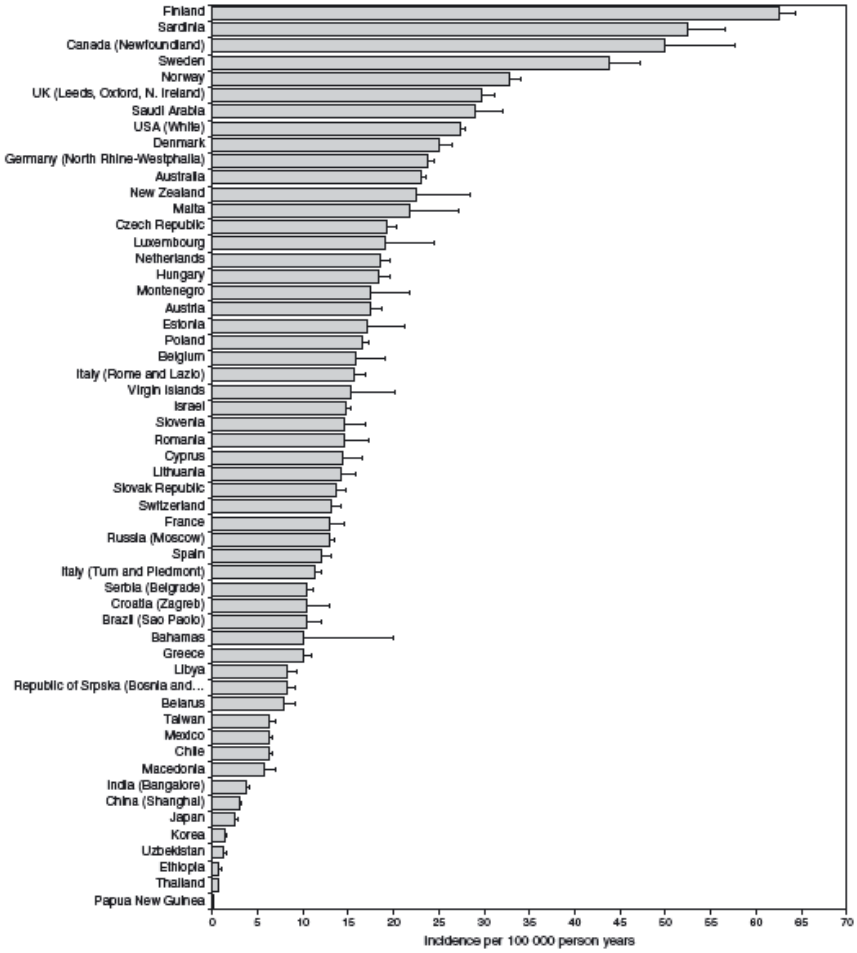

Figure (1): Global mean annual incidence rates of type 1 diabetes in children and adolescents aged 0–14 yr. Only countries in which the study period included data from 2000 onwards are shown [adapted from the International Federation atlas ***(Craig et al., 2014).***

Classification:

WHO classified D.M. into **clinical** (normoglycemia, IGT/IFG, diabetes), and **etiological** types ***(Pickup and Williams, 2003).***

Table (1): Etiological classification of diabetes mellitus ***(ADA, 2014).***

I.	**Type 1 diabetes** (ß-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 ß-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-1 (IPF-1; MODY4)
	5. Chromosome 17, HNF-1ß (MODY5)
	6. Chromosome 2, Neuro D1 (MODY6)
	7. Mitochondrial DNA
	8. Others
	B. Genetic defects in insulin action:
	1. Type A insulin resistance
	2. Leprechaunism
	3. Rabson-Mendenhall syndrome
	4. Lipoatrophic diabetes
	5. Others
	C. Diseases of the exocrine pancreas:
	1. Pancreatitis
	2. Trauma/pancreatectomy
	3. Neoplasia
	4. Cystic fibrosis
	5. Hemochromatosis
	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. Drug- or chemical-induced:

1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. ß-adrenergic agonists
8. Thiazides
9. Dilantin
10. α-Interferon
11. Others

F. Infections:

1. Congenital rubella
2. Cytomegalovirus
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's syndrome
2. Klinefelter's syndrome
3. Turner's syndrome
4. Wolfram's 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 mellitus (GDM)

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, 2007).***

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories:

- Type 1 diabetes.
- Type 2 diabetes ***(ADA, 2007).***

Table (2): Characteristic features of type 1 compared with type 2 diabetes in young people

Characteristics	type 1	type 2
Age	Throughout childhood	Pubertal or later
Onset	Most often acute, rapid	Variable, from slow, mild to severe
Genetics	Polygenic	Polygenic
Race/ ethnics	All groups,but wide variability of incidence	Certain ethnics groups are at particular risk
Frequency	Usually 90%	Most countries 10%
Insulin dependence	Permanent, total, severe	Uncommon, but insulin required when oral hypoglycemic fail
Insulin secretion	Absent or very low	Variable
Insulin sensitivity	Normal	Decreased
Autoimmunity	Yes	No
Ketosis	Common	Rare
Obesity	No	Strong
Acanthosis Nigerians	No	Yes

(Ramin and David, 2004)

Type 1 Diabetes Mellitus:

Type 1 diabetes is generally thought to be precipitated by an immune-associated, if not directly immune-mediated, destruction of insulin-producing pancreatic β cells ***(Todd, 2010).***

Historically, type 1 diabetes was largely considered a disorder in children and adolescents, but this opinion has changed over the past decade, so that age at symptomatic onset is no longer a restricting factor ***(Leslie, 2010).***

Polydipsia, polyphagia, and polyuria (the classic triad of symptoms associated with disease onset) along with overt hyperglycaemia remain diagnostic hallmarks in children and

adolescents, and to a lesser extent in adults. An immediate need for exogenous insulin replacement is also a hallmark of type 1 diabetes, for which lifetime treatment is needed ***(Atkinson et al., 2014).***

Type 1 diabetes classification:

- ***Immune-mediated diabetes (type 1a):***

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β- cells of the pancreas ***(American Diabetes Association (ADA), 2014).***

Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected ***(Watkins et al., 2014).***

Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective ***(American Diabetes Association (ADA), 2014).***

- ***Idiopathic diabetes (Type 1b):***

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes falls into this category, of those who do, most are of African or Asian ancestry ***(American Diabetes Association(ADA), 2014).***

Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go ***(Usher-Smith et al., 2011).***

- ***Fulminant type 1 diabetes (type 1c):***

A new subtype of type 1 diabetes **(Fulminant type 1 diabetes)** was established in the year 2000. It is a syndrome characterized by a markedly rapid and almost complete destruction of pancreatic β cells. Several lines of evidence suggest that both genetic factors, such as human leukocyte antigen (HLA), and environmental factors, such as viral infection, contribute to the development of this disease ***(Koltin and Daneman, 2008).***

Clinical characteristics of Fulminant type 1 diabetes *(Imagawa and Hanafusa, 2006):*

1. 20% of acute onset type 1 diabetes in Japan.
2. Duration of the disease less than 7 days.
3. High plasma glucose levels with near normal HbA1c.
4. Disease onset accompanied by DKA.
5. No C-peptide.
6. Elevated serum pancreatic enzyme level.
7. Negative Anti – islet autoantibodies.

Table (3): The clinical and biological characteristic of 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 levels at diagnosis	↑↑	↑↑	↑↑↑
HbAlc at diagnosis	↑↑	↑↑	Normal or slightly elevated

(Imagawa et al., 2000)