ESTIMATION OF $CD4^+$, $CD25^+$ $FOXP3^+$ CELLS IN CHILDREN WITH TYPE 1 DIABETES

Thesis

Submitted for Partial Fulfillment of Master Degree in Pediatrics

Submitted By

Mohamed Samir Mohamed Ragheb
M.B.,B.Ch.

Under Supervision of

Prof. Dr. Mona Abdel Kader Salem
Professor of Pediatrics
Faculty of Medicine -Ain Shams University

Prof. Dr. Mohamed Aboelasrar Mohamed
Professor of Pediatrics
Faculty of Medicine -Ain Shams University

Dr. Dalia Mohamed Ibrahim
Lecturer of Pediatrics
Faculty of Medicine -Ain Shams University

Faculty of Medicine
Ain Shams University
2012

List of Contents

List of Tables

List of Tables (Cont..)

List of Figures

List of Figures (Cont..)

Page No.	Title	Figure No.

List of Abbreviations

ADA.....	American Diabetes Association
APCs.....	Antigen Presenting Cells
BMI.....	Body Mass Index
BSA.....	bovine serum albumin
CD.....	Cluster of Differentiation
CTLA-4.....	Cytotoxic T lymphocyte associated factor-4
DC.....	Dendritic Cell
DCCT.....	Diabetes Control and Complications Trial
DKA.....	Diabetic Ketoacidosis
DM.....	Diabetes Mellitus
DNA.....	Deoxyribonucleic acid
DPT-1	Diabetes Prevention Trial-1
EAE.....	Experimental autoimmune encephalomyelitis
ENDIT	The European Nicotinamide Diabetes Intervention Trial
Foxp3.....	Forkhead box protein 3
FPG.....	Fasting plasma glucose
GAD65.....	Glutamic Acid Decarboxylase isoform 65
GALT.....	Gutassociated lymphoid tissue
GDM.....	Gestational diabetes mellitus
GITR.....	Glucocorticoid-induced TNF receptor
GRAIL	Gene related to anergy in lymphocytes
Hb	Hemoglobin
HbA1c.....	Glycosylated Hemoglobin
HDL	High density lipoprotein
HLA	Human Leucocytic Antigen
IA2	Insolinoma Associated Antigen -2
IAA	Insulin Auto-Antibody
IBD	Inflammatory bowel disease
ICA	Islet Cell Antibody
IDDM.....	Insulin Dependant Diabetes Mellitus
IFG.....	Impaired fasting glucose
IFN.....	Interferon
IGT.....	Impaired Glucose Tolerance
IL.....	Interleukin
IPEX	Immune dysregulation, polyendocrinopathy, Enteropathy X-linked syndrome
ISPAD.....	International Society for Pediatric and Adolescent Diabete
IVGTT	Intra Venous Glucose Tolerance Test
Jak.....	Janus kinase

List of Abbreviations (Cont…)

Kip1	Cyclin-dependent kinase inhibitor p27
LDL.....	Low density lipoprotein
MAP.....	Mitogen-activated protein
MHC	Major Histocompitabilty Complex
MODY	Maturity-Onset Diabetes of the Young
MRBG.....	Mean random blood glucose
MS.....	Multiple Sclerosis
NK.....	Natural killer
NKC.....	Natural Killer Cells
NKT	Natural killer T cells
NOD.....	Non-Obese Diabetic
OGTT.....	Oral Glucose Tolerance Test
PI3K.....	Phosphatidylinositol 3-kinase
PTPN22.....	Phosphatase non-receptor type 22
RA.....	Rheumatoid arthritis
RBCs.....	Red Blood Corpuscles
RNA.....	Ribonucliec acid
SD	Standered Deviation
SLE	Systemic Lupus Erythematosus
SMBG.....	Self Monitoring Blood Glucose
T1D.....	Type 1 Diabetes
TCR.....	T cell Receptor
TGF.....	Transforming growth factor
Th1&Th2	T helper 1&T helper 2
TLC.....	Total Leukocytic Count
TNF.....	Tumor Necrosis Factor
Treg.....	T regulatory cell
VNTR	variable numbers of tandem repeat
WHO.....	World Health Organization
Wt	Weight

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative deficiency of insulin, an anabolic hormone**.** Insulin is produced by the beta cells of the islets of Langerhans located in the pancreas, and the absence, destruction, or other loss of these cells results in type1 diabetes ***(Lamb, 2011).***

Most diabetic cases fall under the Type 2 category, characterized by relatively late onset, development of insulin resistance and/or deficiency, and amyloidosis. Type 1 diabetes, on the other hand, manifests early during childhood and has an autoimmune component to it that causes a severe deficiency in the circulating levels of insulin. Despite the heterogeneity in etiology and clinical presentation, hyperglycemia is the most common metabolic abnormality in diabetic patients ***(Lee and Pervaiz ,2007).***

Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing pancreatic β cells by autoreactive T cells. Self-reactive CD4+ and CD8+ T lymphocytes infiltrate the pancreas (insulitis) and selectively destroy the insulin producing beta-cells in the islets. This destruction occurs 'silently and progressively' and may stay undetected for many years ***(Chentoufi et al., 2008).*** Susceptible genes and environmental factors are important in the disease process, but the pathological mechanisms are still unknown

(Knip et al., 2005). Failure of immunoregulatory cells in maintaining peripheral tolerance to β cells is one plausible hypothesis ***(Piccirillo & Thornton, 2004).***

T-cell tolerance is established centrally in the thymus and further strengthened and maintained through multiple mechanisms of peripheral tolerance ***(Walker and Abbas, 2002).*** An interest has focused on a feature of tolerance that seems to bridge the central and peripheral processes, namely the $CD4^+CD25^+$ regulatory T-cell ***(Sakaguchi, 2000).*** T regulatory cells modulate a response to autoantigens and probably play a role in pathogenesis of type 1 diabetes ***(Bluestone et al., 2008).***

Naturally occurring regulatory T cells arise during the normal process of maturation in the thymus ***(Dejaco et al, 2006).*** $CD4^+CD25^+$ cells can be detected in peripheral blood in humans and are able to suppress proliferation and cytokine production from both CD4 and CD8 T-cells in vitro in a cell contact–dependent manner ***(Piccirillo and Shevach , 2001).***

Although, islet infiltrates have shown the presence of cytotoxic effector T-cells and pro-inflammatory cytokines ***(Donath et al., 2003).*** There is still a major void in our understanding of how these effector cells escape peripheral regulation ***(Jailwala et al., 2009).***

There is accumulating evidence of a deficiency in either the frequency or function of regulatory T-cells in various human autoimmune diseases ***(Bacchetta et al., 2007).***

Results concerning the role of T regulatory cells in the pathogenesis of diabetes are diverse. Further more both the experimental and clinical studies are required including the use of those cells in immunotherapy. Most authors observed the lack of number and/or function of T regulatory cells in type 1 diabetes ***(Luczyński et al., 2009).***

AIM OF THE STUDY

To evaluate the contribution of T regulatory cells in the pathogenesis of type 1 diabetes, through determination of the count and percentage of $CD4^{+}CD25^{+}$ in the blood.

Hypothesis

It is hypothesized that a relative defect of the number of regulatory T-cells (CD4+CD25) is estimated in type 1 diabetes.

DIABETES MELLITUS

Definition:

Diabetes Mellitus (DM) 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 various organs, especially the eyes, kidneys, nerves, heart and blood vessels ***(American Diabetes Association, 2010).*** It is the most common endocrine – metabolic disorder of childhood and adolescence ***(Sperling, 2000).***

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).***

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.

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

Continued

- *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

Continued

7. Thiazides
8. Dilantin
9. α-Interferon
10. 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)

(ADA, 2007).