



Association of Cytotoxic T Lymphocyte Antigen 4 Gene Polymorphism with Type 1 Diabetes in Children

*Thesis proposal Submitted for fulfilment of PhD in childhood studies
(Child Health and Nutrition)
Medical Studies Department*

By

Marwa Wageeh Mohamed Ali Abou El Naga

M.Sc Paediatrics- Ain Shams University

Under Supervision of

Dr. Randa Kamal Abdel Raouf

Professor of Paediatrics

Department of Medical Studies

Institute of Postgraduate Childhood Studies

Dr. Mona Hussein El Samahy

Professor of Paediatrics

Faculty of Medicine

Ain Shams University

Dr. Hesham Wahid Eldin Mostafa

Professor of Child Health

Department of Child Health

National Research Centre

Dr. Khalda Said Mohamed Amr

Professor of Medical Molecular Genetics

Department of Medical Molecular Genetics

National Research Centre

***Institute of Postgraduate Childhood Studies
Ain Shams University***

2017

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقُلْ رَبِّ زِدْنِي عِلْمًا

صدق الله العظيم

سورة طه الآية (١١٤)

Acknowledgement

First and foremost, thanks to Allah, the most generous, the most merciful.

This work would not have been possible without support of so many people who were always there when I needed them.

*I would like to express my sincere gratitude to **Prof. Randa Kamal Abdel Raouf**, Professor of Pediatrics, Institute of Postgraduate Childhood Studies Ain Shams University, not only for her valuable supervision and great help, but also for her constant support, encouragement and patience, as well as an endless help, support and sympathy during this work.*

*I would like to express my deepest gratitude to **Prof. Mona Hussein El Samahy** Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her valuable experience and for her generous guidance, supervision, kindness, and support.*

*I am greatly indebted and grateful to **Prof. Hesham Wahid Eldin Mostafa**, Research Professor of child health, national research Centre, for his outstanding effort and unlimited co-operation in supervising this work, and for his valuable advice and encouragement as well as an endless help, support and sympathy during this work.*

*I would like to thank **Prof. Khalda Said Mohamed Amr** , Professor of Medical Molecular Genetics , Department of Medical Molecular Genetics , National Research Centre for her invaluable help and for always being so kind, helpful and motivating.*

*I would like to express my deepest gratitude to **Prof. Manal Mansour Abdel Rahman** Research Professor of Child Health, National Research Centre, for her generous guidance, supervision, kindness, special advises and support.*

No words could express my deepest and unlimited indebtedness and love to my father, mother, mother in law, my brothers, my sister and their families who offered me all the help and encouragement that made this work possible.

Special thanks to my dear husband for his great help throughout the whole work, I wish to express my thanks and love to my lovely kids who encourage me to finish this work,

Furthermore, I would like to convey my special thanks to all the patients, their parents and staff members in the Pediatric diabetology clinic of the pediatric hospital of Ain Shams University for their generous co-operation.

ABSTRACT

Background: Type-1 diabetes mellitus (T1DM) is a progressive complex autoimmune disease in which combinations of environmental as well as genetic factors contribute to T-cell mediated destruction of insulin-secreting β -cells of the pancreas. The (CTLA-4) encodes of the T cell receptor involved in the control of T cell proliferation and mediates T cell apoptosis. The contribution of CTLA-4 gene variants to type 1 diabetes has been analyzed in several ethnic groups.

The aim of this Cross sectional, case-control study was to investigate the association of CTLA-4 gene exon 1 49 A/G polymorphism, with T1DM in children and its relation to diabetic complications.

Subjects and methods: A total of 100 subjects were included in the study, those subjects were classified into two groups. Fifty children with T1DM aged 10-18 years (12.5 ± 2.0 years), and fifty healthy age and sex matched children as a control group. All candidates were subjected to full clinical evaluation and anthropometric measurements. All the patients had the following laboratory investigation been done (RBG, average HbA1c, Quantitative determination of urinary microalbumin). CTLA-4 gene polymorphism PCR-RFLP was done for all the subjects included in the study.

Results: In the current study, CTLA-4 genotyping among the diabetic group was: the mutant homozygous genotype GG in 15(30%), the mutant heterozygous genotype AG in 29(58%) and wild homozygous genotype AA in 6(12%). However, among the control group: it was 3(6%) with GG genotype, 19(38%) with AG genotype and 28(56%) with AA genotype with P value < 0.001 which denoting a higher prevalence of AG and GG genotype in diabetic group with highly statistical significance. There was a significant association between CTLA-4 mutant genotypes and patients with younger age of onset of diabetes ($P=0.011$) and higher dose of insulin ($P=0.002$). CTLA-4 +49 mutant genes did not have any impact on complications of type 1 diabetes. Neither has it shown an impact on HbA1c.

Conclusion: The results of the present study shows that the CTLA-4 A/G +49 polymorphism was associated with type 1 diabetes in Egyptian children with a significant association between CTLA-4 mutant genotypes and patients with younger age of onset of diabetes and higher dose of insulin. However, this polymorphism did not have any impact on complications of type 1 diabetes.

Key Word: T1DM, CTLA- 4, Antigen, Polymorphism

Table of Contents

Item	Page
List of abbreviation.....	I
List of tables	IV
List of figures.....	VII
Introduction	1
Aim of the Study	4
Review of Literature:.....	
Chapter 1: Type 1 Diabetes Mellitus.....	5
- Epidemiology	5
- Classification.....	9
- Pathophysiology	11
- Diagnosis	13
- Complications	15
- Prevention	28
- Management.....	30
Chapter 2: Cytotoxic T Lymphocytes Antigen (CTLA4)	50
Chapter 3: Diabetes and Genes	86
Subjects and Methods	99
Results	112
Discussion.....	134
Summary	146
Conclusion	150
Recommendations	151
References	152
Appendix:	
Appendix I Protocol	217
Appendix II Case Sheet.....	234
Arabic Summary.....	

List of Abbreviations

2-h PG	2-hours Post Glucose
ACE	Angiotensin Converting enzyme
ADA	American Diabetes Association
AIRE	The autoimmune regulator
AP2	Adapter protein
APC	Antigen Presenting Cells
ARF-1	ADP ribolysation factor-1
BG	Blood glucose
BMI	Body mass index
BP	Blood Pressure
CD	Cluster of differentiation
cdk4	Cyclin Dependent Kinase 4
CNS	Central nervous system
CSII	Continuous subcutaneous insulin infusion
CTLA-4	Cytotoxic T lymphocyte Antigen- 4
CVD	Cardiovascular disease
DC	Dendritic Cells
DCCT	Diabetes Control and Complications Trial
DCs	Dendritic cells
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
DR	Diabetic retinopathy
flC1LA-4	Full length form of CTLA-4
FOXP3	The transcription factor forkhead box P3
FPG	Fasting plasma glucose
GAD	Glutamic acid decarboxylase
GDM	Gestational Diabetes Mellitus
GFR	Glomerular filtration rate
GI	Glycemic inde
GL	Glycemic load
HbA1C	Glycosylated hemoglobin (Hemoglobin A1c)
HHS	Hyperglycemic hyperosmolar state
HLA	Human Leukocyte Antigen
HNF	Hepatocyte nuclear factor
IA	Islet antigen
IAAs	Insulin autoantibodies
ICA	Including islet cell
ICOS	Inducible co-stimulator

IDDM12	Insulin Dependent Diabetes Mellitus 12
IDF	International Diabetes Federation
IDO	Indoeamine 2,3 Dioxygenase
Ig	Immunoglobulin
IL	Interleukin
INF- γ	Interferon gamma
INS	Insulin gene
ISPAD	International Society for Pediatric and Adolescent Diabetes
IT AMs	Immunoreceptor tyrosine-based activation motifs
IZS	Insulin Zinc Suspension
LAT	Linker of activation in T cells
LAX	Linker for activation of X cells
LCK	Lymphocyte-specific protein tyrosine kinase
liCTLA-4	ligand- independent CTLA-4
LYP	Lymphoid tyrosine phosphatase
MDI	Multiple Daily Injections
MHC	Major Histocompatibility Complex
MODY	Maturity onset diabetes of the young.
NAFLD	Non-alcoholic fatty liver disease
NKT	Natural Killer T cells
NOD	Non-obese diabetic
NPH	Neutral Protamine Hagedorn insulin
NSGP	National Glycohemoglobin Standardization Program
OGTT	Oral Glucose Tolerance Test
PLD	Phospholipase D
PP2A	Protein phosphatase 2A
PTK	Protein tyrosine kinase
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
PVD	Peripheral vascular disease
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
sCTLA-4	Soluble CTLA-4
SIT	SHP2 interacting TRAP
SLE	Systemic Lupus Erythematosus
SNPs	Single nucleotide polymorphisms
T1DM	Type 1 Diabetes Mellitus
Tconv	Conventional T-cells
TCR	T-cell receptor
TCR ζ	TCRzeta
TDD	Total Daily Dose

TDEI	Total daily energy intake
TGF- β	Transforming growth factor beta
TGN	Trans-Golgi network
Th	T helper
TNF	Tumor necrosis factor
TRAPs	Transmembrane adaptor proteins
Treg	Regulatory T-cells
TRIM	T-cell receptor-interacting molecule
USDA	The United States Department of Agriculture

List of Tables

Table No.	Description	Page
Table (1)	Etiologic Classification of Diabetes Mellitus	9
Table (2)	Criteria for the diagnosis of type 1 diabetes mellitus	14
Table (3)	International clinical DR disease severity scale	22
Table (4)	Neuropathies in diabetes	24
Table (5)	Characteristic features of youth onset type1 diabetes in comparison with type 2 diabetes and monogenic diabetes.	27
Table (6)	Types of insulin preparations and suggested action profiles	32
Table (7)	Demographic and diabetic characteristics among diabetic patients	112
Table (8)	Frequency of presenting symptoms of diabetes among diabetic patients	114
Table (9)	Age, Gender, BMI and a Family history of diabetes mellitus among control group	114
Table (10)	Comparison between diabetic patients and controls as regards age, gender, BMI and a family history of diabetes mellitus	115

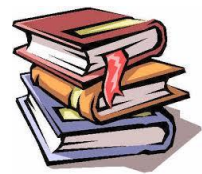
Table No.	Description	Page
Table (11)	Vital data and Laboratory findings among diabetic patients	116
Table (12)	Vital data and Laboratory findings among control group	117
Table (13)	Comparison between diabetic patients and controls regarding blood pressure, pulse rate and Respiratory rate	118
Table (14)	Comparison between Diabetic patients and control groups regarding RBS, HA1C and Microalbuminuria	119
Table (15)	CTLA4 genotypes among diabetic patients	120
Table (16)	CTLA4 genotypes among control group	121
Table (17)	Comparison between diabetic patients and control group regarding distribution of CTLA4 genotypes and alleles	122
Table (18)	Comparison between CTLA4 genotypes regarding demographic characteristics among diabetic patients	123
Table (19)	Comparison between mutant and wild genes regarding demographic characteristics among diabetic patients	124

Table No.	Description	Page
Table (20)	Comparison between CTLA4 genotypes regarding clinical characteristics among diabetic patients	125
Table (21)	Comparison between mutant and wild genes regarding clinical characteristics among diabetic patients	126
Table (22)	Comparison between CTLA4 genotypes regarding vital data among diabetic patients	128
Table (23)	Comparison between mutant and wild genes regarding vital data among diabetic patients	128
Table (24)	Comparison between CTLA4 genotypes regarding laboratory findings among diabetic patients	129
Table (25)	Comparison between mutant and wild genes regarding laboratory findings among diabetic patients	129
Table (26)	Association between alleles and other factors among diabetic patients	130

List of Figures

Figures No.	Figure description	Page
Figure (1)	Incidence and prevalence of type 1 diabetes mellitus in children around the world	7
Figure (2)	Pathogenesis of type 1 diabetes	13
Figure (3)	Food pyramid	39
Figure (4)	Healthy eating plate	43
Figure (5)	CTLA-4 is an inhibitory receptor for B7	70
Figure (6)	Proposed mechanisms by which CTLA-4 inhibits T-cell activation.	76
Figure (7)	The 4 major genes associated with type 1 diabetes	90
Figure (8)	Mechanism-of-action-of-ipilimumab	98
Figure (9)	DNA double helix	107
Figure (10)	Diabetic complications among diabetic patients	113
Figure (11)	Comparison between diabetic patients and control group regarding family history of DM	116
Figure (12)	Microalbuminuria among study groups	119
Figure (13)	Comparison between diabetic patients and control group regarding genotypes of CTLA4 gene	123
Figure (14)	Comparison between different genotypes regarding age of onset among diabetic patients	127

Figure (15)	Comparison between different genotypes regarding insulin dose among diabetic patients	127
Figure (16)	Agarose gel electrophoresis (2%) stained with ethidium bromide showing the CTLA-4 genotyping	131
Figure (17)	Agarose gel electrophoresis showing CTLA4 49 A/G genotypes of T1DM patients after BbvI digestion-1	132
Figure (18)	Agarose gel electrophoresis showing CTLA4 49 A/G genotypes after BbvI digestion-2	133



INTRODUCTION
