Study of Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) mRNA Expression in Patients with Systemic Lupus Erythematosus

A Thesis

Submitted for Partial Fulfillment of MD Degree
In
Clinical and Chemical Pathology

By
Mona Mohsen Ahmed Abou ElGhar
M.Sc. Clinical and Chemical Pathology
Cairo University

Supervised By

Prof. Dr. Safaa Mostafa ElKaraksy
Professor of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Prof. Dr. Noha Mohamed Hosny Shahin
Professor of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Prof. Dr. Naglaa Mohamed Kholoussi
Professor of Clinical and Chemical Pathology

National Research Center

Ass. Prof. Dr. Rasha El-Sayed Gheith
Assistant Professor of Rheumatology and Rehabilitation
Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University
2014


بسم الله الرحمن الرحيم

ACKNOWLEDGEMENT

First of all, praise to God the most generous for enabling me to conduct this study. I would like to thank Professor Doctor **Safaa Mostafa ElKaraksy** *Professor of Clinical and Chemical Pathology Cairo University for her wise guidance and co-operation throughout the of the study. Without her genuine advice and understanding, this work would have never been complete.*

My appreciation is due to Professor Doctor **Naglaa Mohamed Kholoussi** *Professor of Clinical and Chemical Pathology National Research Center for her kind and valuable help. My deepest gratitude to Professor Doctor* **Noha Mohamed Hosny Shahin**, *Professor of Clinical and Chemical Pathology Cairo University, whose sincere effort and devotion were a cornerstone to this study.I would also like to thank Assistant Prof Dr* **Rasha El-Sayed Gheith**, *Professor of Rheumatology and Rehabilitation Cairo University, for her co-operation and assistance. She supplied me with valuable information which was important pillars in my thesis.*

My sincere appreciation to Dr **Rasha Shahin** *Lecturer of Clinical and Chemical Pathology Cairo University. She kindly guided my steps and was always providing me with valuable suggestions, advice, experience and constant support which were the cornerstone in the completion of this study. She was very keen and helpful.*

ABSTRACT

TRAIL (APO-2 ligand) is a transmembrane (type II) glycoprotein which also belongs to the TNF superfamily.

TRAIL triggers apoptosis through interaction with the death receptors Death Receptor (DR4) (TRAIL-R1) and (DR5) (TRAIL-R2).

There are several indications that TRAIL could be involved in the pathophysiology of autoimmune diseases in general and systemic lupuserythematosus (SLE) in particular.

The aim of this study was to investigate TRAIL m RNA expression levels in peripheral blood mononuclear cells(PBMCs) from patients with SLE and to study the association between the results and various clinical and laboratory data of the patients in order to assess the possible role of TRAIL in SLE pathogenesis and its relation to disease activity.

In conclusion, our study revealed higher expression levels of the TRAIL mRNA in PBMCs from SLE patients compared to those from healthy controls.

Key words: Tumor necrosis factor related apoptosis inducing ligand, Systemic lupus erythematosus, Real time PCR.

TABLE OF CONTENTS

	Page
List of Abbreviations	III
List of Figures	V
List of Tables	VI
INTRODUCTION AND AIM OF WORK	1
REVIEW OF LITERATURE	
▪ **CHAPTER I:** Systemic Lupus Erythematosus	3
▪ **CHAPTER II:** Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL)	25
SUBJECTS AND METHODS	40
RESULTS	53
DISCUSSION	69
SUMMARY AND CONCLUSIONS	74
REFERENCES	76
APPENDIX	85
ARABIC SUMMARY	

LIST OF ABBREVIATIONS

AIF	:	Apoptosis Inducing Factor
ANA	:	Antinuclear Antibody
Anti DNA	:	Anti DeoxyRibonucleic Acid Antibody
Apaf-1	:	Adaptor Protein
APC	:	Antigen Presenting Cell
BILAG	:	British Isles Lupus Assessment Group
BLyS	:	B-lymphocyte Stimulator
C 1	:	Complement Number 1
C 2	:	Complement Number 2
C 3	:	Complement Number 3
C 4	:	Complement Number 4
CNS	:	Central Nervous System
CR	:	Complement Receptor
CRP	:	C-reactive Protein
CSF	:	Cerebrospinal Fluid
CTLs	:	Cytotoxic T lymphocytes
CVA	:	Cerebrovascular Accidents
cyto c	:	Cytochrome C
DCs	:	Dendritic Cells
DR4	:	Death Receptor 4
EAE	:	Experimental Autoimmune Encephalomyelitis
EBV	:	Epstein Barr Virus
EC4d/CR1	:	Erythrocyte Complement Receptor 1
ECG	:	Echocardiography
ELISA	:	Enzyme-linked Immunosorbent Assay
EndoG	:	Endonuclease G
FADD	:	Fas-Associated Death Domain
GADPH	:	Glyceraldehydes 3-phosphate dehydrogenase
HLA	:	Human Leucocyte Antigen
IL	:	Interleukin
KPCR	:	Kinetic Polymerase Chain Reaction
LPS	:	Lipopolysaccharide

MGPs	:	Magnehic Glass Particles
MHC	:	Major Histocompatibility Complex
MS	:	Multiple Sclerosis
NF	:	Nuclear Factor
OPG	:	Osteoprotegerin
PBMC	:	Peripheral Blood Mononuclear Cells
PBS	:	Phosphate-Buffered Saline
PCR	:	Polymerase Chain Reaction
PMNs	:	Polymorphnuclear cells
RIA	:	Radioimmunoassay
RT-PCR	:	Real-Time Reverse Transcription-Polymerase Chain reaction
SCID	:	Severe Combined Immunodeficiency
SLAM	:	Systemic Lupus Activity Measure
SLE	:	Systemic Lupus Erythematosus
SLEDAI	:	Systemic Lupus ErythematosusDisease Activity Index
Sm	:	Smith
SMAC	:	Second Mitochondrial-Derived Activator ofcaspase
SPSS	:	Statistical Package For The Social Science
TCR	:	T Cell Receptor
TGFB	:	Transforming Growth Factor B
Th	:	T Helper Cells
TNF	:	Tumor Necrosis Factor
TRAIL	:	Tumor Necrosis Factor- Related ApoptosisInducing ligand
UV light	:	Ultraviolet

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Diagram summarizing clinical criteria of systemic lupus erythematosus	**14**
2.	Crithidialucilia (positive DNA)	**20**
3.	Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors	**26**
4.	Pathway of TRAIL	**30**
5.	Schematic diagram illustrating the TRAIL and apoptosis signaling	**32**
6.	Cytotoxic CD8+ T cells lyse tumours through death ligands	**34**
7.	Magna Pure compact nucleic acid Isolation instrument	**44**
8.	Magna Pure isolation kit	**44**
9.	Frequency and percentage of each sex in SLE Patients	**60**
10.	Clinical data of SLE Patients as regards Fever	**60**
11.	Clinical data of SLE Patients as regards Alopecia	**61**
12.	Clinical data of SLE Patients as regards Rash	**61**
13.	Clinical data of SLE Patients as regards Arthralgia and arthritis	**62**
14.	Clinical data of SLE Patients as regards Myositis	**62**
15.	Clinical data of SLE Patients as regards Lupus nephritis	**63**
16.	Clinical data of SLE Patients as regards Serositis	**63**
17.	Clinical data of SLE Patients as regards Lupus cerebritis	**64**
18.	Clinical data of SLE Patients as regards Vasculitis	**64**
19.	Frequency and percentage of SLEDAIin SLE patients .	**65**
20.	Laboratory data of SLE patients as regards complement consumption .	**65**
21.	Laboratory data of SLE patients as regards positive ANA	**66**
22.	Laboratory data of SLE patients as regards positive anti ds DNA	**66**
23.	Target amplifications curves of TRAIL mRNA expression in SLE patients	**67**
24.	Standard curve	**68**

LIST OF TABLES

Table No.	Title	Page No.
1	The genetic components associated with SLE	**4**
2	T-cell subsets and cytokines	**9**
3	The 1982 revised criteria for classification of SLE	**12**
4	Some indicators of increased risk of disease damage	**18**
5	The weight in SLEDAI score column	**23**
6	the 20 µl PCR reaction mixture for TRAIL amplification included mixture of the following components	**49**
7	Clinical data of studied SLE patients	**56**
8	Routine Laboratory data of SLE patients	**57**
9	Special Laboratory data of SLE patients	**57**
10	Laboratory data of controls	**57**
11	Relative expression of the TRAIL mRNA in SLE Patients and controls	**57**
12	Statistical comparison between patients and controls as regarding TRAIL mRNA expression	**57**
13	Statistical comparison between high and normal TRAIL expressers in SLE patients regarding clinical data	**58**
14	Statistical Comparison between high and normal TRAIL expressers in SLE patients regarding laboratory data	**59**
15	Clinical data of SLE patients	**85**
16	Laboratory dataof SLE patients	**86**
17	Clinical data of control group	**87**
18	Laboratory data of control group	**88**

INTRODUCTION AND AIM OF WORK

Systemic Lupus erythematosus (SLE) is a disease characterized by flares, remissions and autoantibodies directed against several intracellular and cell-surface antigens. The course of the disease is variable ***(Li and Isenberg, 2006)***. SLE can affect virtually any organ and it frequently involves the skin, joints, heart, lungs, kidneys and central nervous system (CNS) ***(Croker and Kimberly, 2005)***.

Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), is a type II transmembrane protein ***(Rus et al., 2005)***, that modulates the apoptotic response by binding to receptors, including the death receptor 4 (DR4, TRAIL-R1), (KILLER/DR5 ,TRAIL-R2), TRID (DcR1, TRAIL-R3), TRUNDD (DcR2, TRAIL-R4), and osteoprotegerin (OPG) ***(Jung et al., 2005).***

Apoptosis (programmed cell death) is an active physiological process that leads to the ordered destruction of cells without the release of intracellular contents into the extracellular environment (which would cause an inflammatory reaction and tissue damage). It is fundamental to maturation and homeostasis of the immune system .Apoptosis can be induced passively, through lack of essential survival signals, or actively, through ligand induced trimerisation of specific death receptors of the tumour necrosis factor (TNF) receptor family, such as Fas, the TNF receptor or the TNF-related apoptosis inducing ligand (TRAIL)receptor ***(Nagata , 1999)***.

There are several indications that TRAIL could be involved in the pathophysiology of autoimmune diseases in general and SLE in

particular. In patients with multiple sclerosis, FasL and TRAIL are upregulated in peripheral blood mononuclear cells ***(Huang et al.,2000)***. ***Kaplan et al (2002)*** reported that increased expression of TRAIL and FasL found on activated T cells contributes to increased monocyte apoptosis in patients with SLE. Furthermore ***Rus et al.,(2002)*** found increased mRNA for TRAIL and its decoy receptors (TRAIL-R3 and TRAIL-R4) in peripheral blood mononuclear cells from SLE patients.

The aim of this study was to estimate TRAIL m RNA expression levels in PBMCs from patients with SLE and to study the association between these results and various clinical and laboratory data of the patients in order to assess the possible role of TRAIL in SLE pathogenesis and its relation to disease activity.

CHAPTER I

Systemic Lupus Erythematosus

Systemic Lupus erythematosus (SLE) is a chronic and systemic autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors ***(Sánchez et al.,2006).***

The disease is characterized by flares, remissions and autoantibodies directed against several intracellular and cell-surface antigens. The course of the disease is variable and regular review is essential to detect new and recurrent organ/system involvement. However, not all of the antibodies found in SLE are clinically relevant ***(Li and Isenberg, 2006)***.

SLE can affect virtually any organ and it frequently involves the skin, joints, heart, lungs, kidneys and central nervous system (CNS) ***(Croker and Kimberly, 2005)***.

Incidence:

SLE predominantly affects women, especially those of reproductive age, and women of African , American or Hispanic American heritage have a 3–4 times increased risk of developing disease compared to Caucasians. In most patients, SLE develops between the ages of 15 and 45 years. The female: male ratio is at least 9:1. In a minority of patients, disease develops after the age of 50 years, and in this subgroup the female: male ratio is 4:1. In childhood disease, the ratio of girls to boys is 3:1 ***(Reveille et al., 1998).***

Genetic Susceptibility:

The genetic background of SLE is thought to be complex and involves multiple genes encoding different molecules with significant functions in the regulation of the immune system ***(Cantor et al., 2004).*** Among the genetic factors believed to influence susceptibility to SLE, the major histocompatibility complex (MHC) alleles show the most significant association. Importantly, several studies show that non-HLA genes play a role in the development of SLE ***(Prokuninaand Alarcon-Riquelme., 2004).***

The currently known genes and gene regions associated with human SLE are shown in Table (1).

Table (1): The genetic components associated with SLE: ***(Prokunina and Alarcon-Riquelmeal., 2004)***.

Genetic factors	
Complement	*C1,C4 deficiencies (> 75% prevalence, severe disease) *C2 deficiency (~35% prevalence, severity similar to non-C deficient lupus patients) *C4 50-80-% of patients partially deficient CR1 deficiencies (acquired rather than genetic)
MHC association	*HLA-A1, B8,DR3-English/Irish patients *DR3 and DQw2-antiRo/La antibodies *DQw6,7,8-lupus anticoagulant *DR2-Afro-Caribbean patients with anti Sm *DR2-DQw1 (DQw6), DQ2-susceptibility to nephritis *DR4, DQw3-Protective? *DRw8-early onset SLE (<20 years) *Decreased expression of class II on APC *Susceptibility may depend upon a single amino acid substitution (Histidine→ tyrosine at position 30 in sequence of DQw1.19b β gene from single SLE patient compared to healthy control)
T-cell receptor	*AntiRo response related to TCR β gene product in SLE *Genomic DNA restriction fragment length polymorphism in humans No deletions, insertions in TCR α, β, γ chain genes

DNA regions linked to SLE by Genome Scanning: ***(Lee and Nath, 2005).***

- 1q23 (probably contains FcγRIIIA)
- Studies of genome scanning of SLE revealed regions on chromosomes: 1q41-42, 1q25-31, 2q35-37, 4p16-15.2, 6p11-21, 12p24, and 16q12 ***(Tsao, 2004)***.
- Two genomic locations at 6p22.3-6p21.1 and 16p12.3-16q12.2 were identified

Pathogenesis

This heterogeneous disease is caused by the complex interaction of a variety of abnormalities which cause disease susceptibility and/or provoke disease onset or exacerbation. At the core of this process are immune dysfunction and production of autoantibodies ***(Manson and Isenberg, 2003)***.

SLE and autoantibodies:

B lymphocytes from patients with SLE display a lack of self tolerance, and an inappropriate overproduction of antibody. The presence of antinuclear autoantibodies (ANA) is a sensitive test, found in 98% of patients with SLE ***(Worrall et al., 1990)***.

Anti-DNA antibodies are seen in approximately 60% of patients with SLE but the presence of anti-DNA antibodies is the most specific finding. Serial serum concentrations of anti-DNA antibodies reflect disease activity in many patients, but not all as some patients have high anti-DNA antibody levels without overt disease ***(Borg et al., 1990)***.

Instead of simply acting as a disease marker, some anti-DNA antibodies are, in some way, directly pathogenic. For example, studies have shown that injecting human hybridoma-derived anti-DNA antibodies into severe combined immunodeficiency (SCID) mice results, in some cases, in renal deposition of antibody with associated proteinuria***(Ehrenstein et al., 1995)***.

In addition to ANA and anti-DNA antibodies, a variety of other autoantibodies are often detected. The antigens targeted may be associated with patient ethnicity, for example, increased levels of anti-Sm antibodies are seen in Afro-Caribbean patients, or particular disease manifestations, for example, anti-Ro antibodies seen in association with a photosensitive rash.

Finally patients with lupus are often found to have positive antiphospholipid antibodies, with or without the related clinical syndrome ***(Manson and Isenberg, 2003)***.

SLE and B cells and T cells

Central to immune dysfunction seen in SLE is the existence of overactive B cells, which produce an abundance of autoantibody. The development and survival of these cells is dependent upon T-cell help. The propagation of self-directed B-cell clones may be assisted by an inappropriate lack of T-cell suppression. B-cell activators, such as the protein B-lymphocyte stimulator (BLyS), appear to be upregulated in lupus, further encouraging B-cell survival ***(Stohl et al., 2002)***.Powerful evidence for the strength of the role of B cells in disease development comes from a study of B-cell depletion therapy in patients with SLE who