

# داء المقوسات و حالات الإجهاض: التعبير الجيني لسيتوكينات الإلتهاب للجهاز المناعى

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مقدمة من

**الطبيبة / منى سعيد الشربيني**

مدرس مساعد بقسم الطفيليات الطبية - كلية طب قصر العيني

تحت اشراف

**أ.د./ أمانى أحمد عبد العال**

أستاذ علم الطفيليات الطبية

كلية طب قصر العيني- جامعة القاهرة

**أ.م.د./ وليد سيد الشربيني**

أستاذ مساعد أمراض النساء و التوليد

كلية طب قصر العيني- جامعة القاهرة

**د. / سمر سيد عطية**

مدرس علم الطفيليات الطبية

كلية طب قصر العيني- جامعة القاهرة

**د./ محمد شرف الدين زكى بدر**

مدرس علم الميكروبيولوجى و البيولوجيا الجزيئية

مركز تطوير الابحاث الطبية- كلية طب - جامعة عين شمس

كلية طب -جامعة القاهرة

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# **Toxoplasmosis and Abortion: Inflammatory Cytokines Gene Expression of the Host Immune System**

**Thesis**

Submitted in Partial Fulfilment of M.D. degree in Medical Parasitology

By

***Mona Said El-Sherbini***

Assistant Lecturer of Medical Parasitology,  
Faculty of Medicine, Cairo University

**Under Supervision of:**

***Prof. Dr. Amany Ahmed Abd El-Aal***

Professor of Medical Parasitology  
Faculty of Medicine, Cairo University

***Dr. Walid Sayed El-Sherbiny***

Assistant Professor of Obstetrics and Gynaecology  
Faculty of Medicine, Cairo University

***Dr. Samar Sayed Attia***

Lecturer of Medical Parasitology  
Faculty of Medicine, Cairo University

***Dr. Mohammed Sharaf El-Din Zaky Badr***

Lecturer of Microbiology and Molecular Biology  
Center of Medical Research Development, Ain Shams University

**Faculty of Medicine  
Cairo University  
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*“This research work is humbly dedicated first and foremost to Allah Almighty, the Merciful and the Beneficent, for endowing me with the required knowledge and every necessary provision, strength and fortitude to go through accomplishing this study.”*

*Mona*

# Abstract

The main aim of this work was to study gene expression of some important inflammatory cytokines in cases infected with *Toxoplasma* and presenting with repeated abortion in comparison to *Toxoplasma* multiparous women as a control. The work comprised a total of 61 serologically *Toxoplasma* positive women which were divided into; Group1: 19 cases suffering from repeated abortion and failed to complete all pregnancies. Group2: 28 multiparous women. Group3: 14 cases were multiparous and suffering from repeated abortion. For gene expression assay, a quantitative real-time PCR system was optimized using 2 pro-inflammatory cytokines (IFN- $\gamma$  & TNF- $\alpha$ ) and 2 anti-inflammatory cytokines (IL-10 & TGF- $\beta$ ). In general, all the studied cytokines were significantly upregulated in Gp1 with a remarkable bias towards the pro-inflammatory cytokines represented by the IFN- $\gamma$  particularly, denoting a predominantly inflammatory state in Gp1. In Gp2, the 4 cytokines were approximately in a balanced state with a little shift towards the 2 anti-inflammatory cytokines (IL-10 and TGF- $\beta$ ). Besides, a significant positive correlation was reported, indicating a harmony in their expression. In Gp 3, the anti-inflammatory (IL-10) was upregulated; also its negative correlation with the pro-inflammatory cytokine (IFN- $\gamma$ ), denotes the possible role of this regulatory cytokine in saving pregnancies' outcomes in Gp3. Hence, the present study hypothesized a potential role of these cytokines in the upcoming prognostic or/and therapeutic concerns.

**Key words:** *Toxoplasma*, repeated abortion, Gene expression, real-time PCR, pro-inflammatory and anti-inflammatory cytokines.

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## LIST OF ABBREVIATIONS

<	More than
>	Less than
°C	degree celcius
18S	Small subunit:18
3C	Chromosome Conformation Capture
3D	Three dimension
A	Adenine
Ab	Antibody
Ac	Apical complex
ACT	Actin gene
ADLYs	disability-adjusted life years
AIDS	Acquired Immune Deficiency Syndrome
ARE	Arabic Republic of Egypt
BME	$\beta$ -mercaptoethanol
bp	base pair
C	Cytosine nucleotide
CD8	Cluster of differentiation number 8
CDC	Centre of disease control and infection
cDNA	Complementary DNA strand
CMV	Cytomegalovirus
CNS	Central nervous system
Cp	Crossing point
cPCR	Conventional polymerase chain reaction
Ct	Crossing threshold
CTL	Cytotoxic lymphocytes
D.C	Dendritic Cells
dept.	department
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease enzyme
dNTP	deoxyriboNucleotide Triphosphates
ds DNA	double stranded DNA
DSA	DNase stop solution
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbant assay
etc.	E t cetera



EVT	Extravillous trophoblasts
fg	fibrinogen-related procoagulant
fig.	figure
FR	Free-range
G	Guanine nucleotide
g	gravity
GAPDH	glyceraldehyde -3- phosphate dehydrogenase
Gp	group
GTC	Guanidine thiocyanate
h.	hour
H <sub>2</sub> O	water
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCl	Hydrochloric acid
HKG	House keeping Gene
HRP	Horseradish peroxidase
HSV	Herpes simplex virus
IFAT	Indirect fluorescence antibody titre
IFN- $\gamma$	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
ISAGAs	Immunosorbent Agglutination Assays
IU	International unit
KDa	kilo Dalton
Lab.	laboratory
LAK	lymphokine-activated killer cell
M	Mole
MERs	Medium reiteration frequency repetitive sequence
Min	minute
MJ	Moving junction
ml	millilitre
mM	millimole
M-MLV H-	Moloney Murine Leukemia Virus hybrid, reverse transcriptase enzyme
MnCl <sub>2</sub>	Manganese chloride
mRNA	Messenger ribonucleic acid

N	number
NBS	NanoBioSys instrument
ng	nanogram
NK	Natural killer cell
nm	Nano meter
ns	Non- significant
NTC	No template control
OD	Optical density
Oligo-dT	short sequence of deoxy-thymine nucleotides
PBMCs	Peripheral blood mononuclear cells
PCR	polymerase chain reaction
PS	pyrimethamine + sulfadiazine
PSF	pyrimethamine + sulfadiazine + folic acid
PV	Parasitophorous vacuole
P-value	probability value
PVM	Parasitophorous vacuole Membrane
qPCR	Quantitative real-time PCR
rRNA	ribosomal RNA
RDA	RNA dilution buffer(blue buffer)
RDTs	Rapid diagnostic tests
RES	Reticuloendothelial system
RFLP	Restriction fragment length polymorphism
RLA	RNA lysis buffer
RNase	Ribonuclease enzyme
rpm	Round per minute
RT	Reverse transcriptase enzyme
RWA	RNA wash solution
rxn	Reaction mixture equivalent
SAG-1	<i>Toxoplasma</i> surface antigen-1
SAGE	Serial Analysis of Gene Expression
SD	standard deviation
SDS	Sodium dodecyl sulphate
Sec.	second
SPSS	Statistical package for social science
SYN	syncytium

T	Thymine nucleotide
<i>T.</i>	<i>Toxoplasma gondii</i>
Taq	<i>Thermus aquaticus</i>
TGF- $\beta$	Transforming growth factor beta
T <sub>m</sub>	Melting temperature
TMB	Tetra methylbenzidine
TNF- $\alpha$	Tumour necrosis factor alpha
TORCH	Toxoplasmosis, Others (Hepatitis B), Rubella (German measles), Cytomegalovirus (CMV), Herpes Simplex Virus (HSV).
<i>Toxo</i>	<i>Toxoplasma</i>
U	Uracil nucleotide
u NK cells	Uterine natural killer cells
UK	United Kingdom
US	United States
USA	United States of America
UV	ultra violet
v/v	Volume per volume
WB	Western blot
$\beta$ -Actin	Beta actin gene
$\mu$ l	microlitre
$\mu$ m	micrometer
$\mu$ M	micromole

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