Tumor Necrosis Factor-Alpha & Anticardiolipin Antibodies in Women with History of Unexplained Recurrent Miscarriage

Thesis submitted for complete fulfillment of Master degree in Clinical and Chemical Pathology

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Abstract

Unexplained RM accounts for about 50% of the cases of recurrent miscarriage. The study examined the correlation and prevalence of TNF-α and ACA in Egyptian women; with history of unexplained recurrent miscarriage. There was an association between the presence of ACA and unexplained RM, and a highly significant positive correlation between the number of previous miscarriages; and the frequency of ACA IgG positivity was detected. There is no association between TNF-α and unexplained RM.

Keywords:

- Unexplained recurrent miscarriage.
- Tumor necrosis factor-alpha (TNF-α).
- Anticardiolipin antibodies (ACA).

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Contents

List of tables:

List of figures

List of abbreviation

A506G	Adenine 506 Guanine
ACA	Anticardiolipin antibody
ANOVA	Analysis of variance
APAS	Antiphospholipid antibody syndrome
APC	Antigen presenting cell
aPL	Antiphospholipid antibodies
APTT	Activated partial thrombin time
ASA	Antisperm antibodies
β2GPI	Beta 2 glycoprotein I
C3	Complement 3
C677T	Cytosine 677 Thymine
CBC	Complete blood count
CRH	Cortisol releasing hormones
CTB	Cytotrophoblast
DCs	Dendritic cells
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
ETF-3	Embryotrophic factor-3
EVT	Extravillous differentiation
Fas-L	Fas-ligand

G20210A	Guanine 20210 Adenine
G-CSF	Granulocyte-Colony stimulating factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
hCG	Human chorionic gonadotropines
HLA	Human leukocyte antigen
HRP	Horse radish peroxidase
iC3b	Inactivated complement 3 b
ICM	Inner cell mass
IDO	Indoleamine diogenase
IFN-γ	Interferon-gamma
IL	Interleukin
IRM	Idiopathic recurrent miscarriage
IUGR	Intrauterine growth retardation
IVF	Invitro fertilization
KAR	killer cell activating receptor
KIR	killer cell inhibitory receptor
LA	Lupus anticoagulant
LIF	Leukemia inhibitory factor
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-Colony stimulating factor
MHC	Major histocompatibility
MMP-9	Matrix metalloproteinase-9

MTHER	Methyl tetra hydro folate reductase
mTNFR	Membrane bound tumor necrosis factor receptor
NFκB	Nuclear factor kappa B
NK	Natural killer
NSAIDs	Non Steroidal Anti-Inflammatory Drugs
NTD	Neural tube defect
OD	Optical density
PAI-1	Plasminogen activator inhibitor-1
PBMC	Peripheral blood mononuclear cells
PCOS	Polycystic ovary syndrome
PG	Prostaglandin
RM	Recurrent miscarriage
RSA	Recurrent spontaneous abortion
sE-selectin	Soluble E-selectin
sHLA	Soluble human leukocyte antigen
sP-selectin	Soluble P-selectin
SPSS	Statistical package for social science
sTB	Syncytiotrophoblast
sTNF	Soluble tumor necrosis factor
sVCAM-1	Soluble vascular cell adhesion molecule-1
TACE	Tumor necrosis factor alpha converting enzyme
Tc	Cytotoxic T-lymphocyte

TF*	Tissue factor
TGF-β	Transforming growth factor-beta
Th	Helper T-Lymphocyte
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
tPA	Tissue plasminogen activator
TRADD	Tumor necrosis factor receptor associated death domain
TRAF	Tumor necrosis factor receptor associated factors
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
TRAIL-R	TRAIL-receptor
tTG	Tissue transglutaminase
UAP	Uterine activation proteins
uNK	Uterine natural killer
vSMC	Vascular smooth muscle cell
XCI	X chromosome inactivation

Introduction and Aim of Work

Miscarriage is the most common complication of human pregnancy, this is a problem that often places couples under a great deal of emotional distress and poses physicians with a formidable challenge ***(Toshiyuki, 2004)***. Spontaneous miscarriage is defined as the involuntary termination of pregnancy before 20 weeks of gestation (dated from the last menstrual period) or spontaneous expulsion of the fetal weight of 500 gm ***(Speroff and Fritz, 2005)***.

Recurrent miscarriage affects about 1% of reproductive aged women; it is defined as three or more consecutive pregnancy losses before 20 gestational weeks ***(Bricher and Farquharson, 2002)***, however, more than two pregnancy losses, including those which are not consecutive, are also recently considered as recurrent spontaneous abortions. ***(Yuce et al., 2007)***

Although much research has been done into the causes of RM, there is a lack of consensus about it. Indeed, Parental chromosome aberrations and the thrombotic complication of the antiphospholipid antibody syndrome are typically quoted as documented causes. However, collectively these abnormalities account for less than 10-15% of recurrent miscarriages ***(Lee-Fox and Schust, 2007)***, and in about 50% of the cases the causes are unknown. ***(Christensen et al., 2008)***

It is now proposed that cell mediated immunological mechanisms may account for a proportion of unexplained recurrent miscarriage ***(Raghupathy et al., 2002)***, since they influence all steps of reproduction and play a fundamental role in pregnancy outcome. ***(Daher et al., 2003)***

Tumor Necrosis Factor-alpha (TNF-α) is a multifunctional Th-1 cytokine which roles in regulating hormone synthesis, placental architecture and embryonic development and it is also secreted by mononuclear phagocytes and natural killer (NK) cells ***(Berman et al., 2005)***.

Due to its pro-inflammatory and pro-apoptotic capacity, TNF-alpha is described to mediate several aspects of pregnancy complications, including pre-eclampsia ***(Anim-Nyame et al. 2003)***, miscarriage ***(Arslan et al., 2004)*** and RM ***(Rezaei & Dabbagh, 2002)***. The fact that it promotes the proliferation of lymphocytes in some cases and, in other cases, forces them into apoptosis, makes its role such a diverse and often contradictory. ***(Goodsell, 2006)***

Another important immunological factor is the anticardiolipin antibodies, women who are positive for anticardiolipin antibodies are at higher risk for recurrent miscarriage ***(Gaber, 2004)***. Anticardiolipin antibodies are circulating autoantibodies immunoglobulin G (IgG) and Immunoglobulin M (IgM) directed essentially against cardiolipin, which is a negatively charged phospholipid. Anticardiolipin antibodies may exert a direct pathogenic effect by interfering with haemostatic processes that take place on the phospholipid membranes of cells such as platelets or endothelium. It is also suggested that autoantibodies may cause intravascular coagulation leading to recurrent abortion. ***(Daboubi, 2001)***

Aim of Work:

To investigate the prevalence and correlation of immunological factors in the studied Egyptian population of women with unexplained recurrent miscarriage, namely:

a- Tumor Necrosis Factor-alpha (TNF-α).

b- Anticardiolipin antibodies (ACA) IgM and IgG.

Chapter 1: Recurrent miscarriage

The maximum probability of conceiving during a menstrual cycle is only about 40%. The vast majority of continuing pregnancies result in the birth of a healthy human being who will, eventually, pass his or her genes on to the next generation. Miscarriages—clinically detectable pregnancies that fail to progress—are the inevitable byproduct of such a process. They are common and often remain unexplained, even after investigation. They are a source of distress for women and their partners ***(Wang et al., 2003)***.

❖ Definitions & epidemiology:

Spontaneous miscarriage is defined as the involuntary termination of pregnancy before 20 weeks of gestation (dated from the last menstrual period) or spontaneous expulsion of the fetal weight of 500 gm ***(Speroff and Fritz, 2005)***. 10 – 20 % of all clinically recognized pregnancies end in spontaneous abortion. However, the actual incidences may be greater when early unconfirmed pregnancies are included ***(Bagratee et al., 2004)***.

Recurrent miscarriage (RM) is defined as three or more consecutive pregnancies ending spontaneously before the 20^{th} week of gestation ***(Bricher and Farquharson, 2002)***. However, more than two pregnancy losses, including those which are not consecutive, are also recently considered as recurrent spontaneous abortions ***(Yuce et al., 2007)***.

Recurrent first trimesteric abortion is defined as the occurrence of three or more clinically recognized losses before 12 weeks from last menstrual period and it affects about 15% of all pregnancies ***(Weeks and Gemzell, 2006)***.

❖ Risk factors:

a. Age:

Advanced maternal age is the most common cause of spontaneous miscarriage in healthy women ***(Figure-1)***. The overall rate of spontaneous miscarriage was (11%) and the approximate frequencies of clinically recognized miscarriage according to maternal age were: age 20-30 years 9-17%, age 30-35 years 20%, age 35-40 years 40% and age over 40 years 80% ***(Nybo Andersen et al, 2000)***.

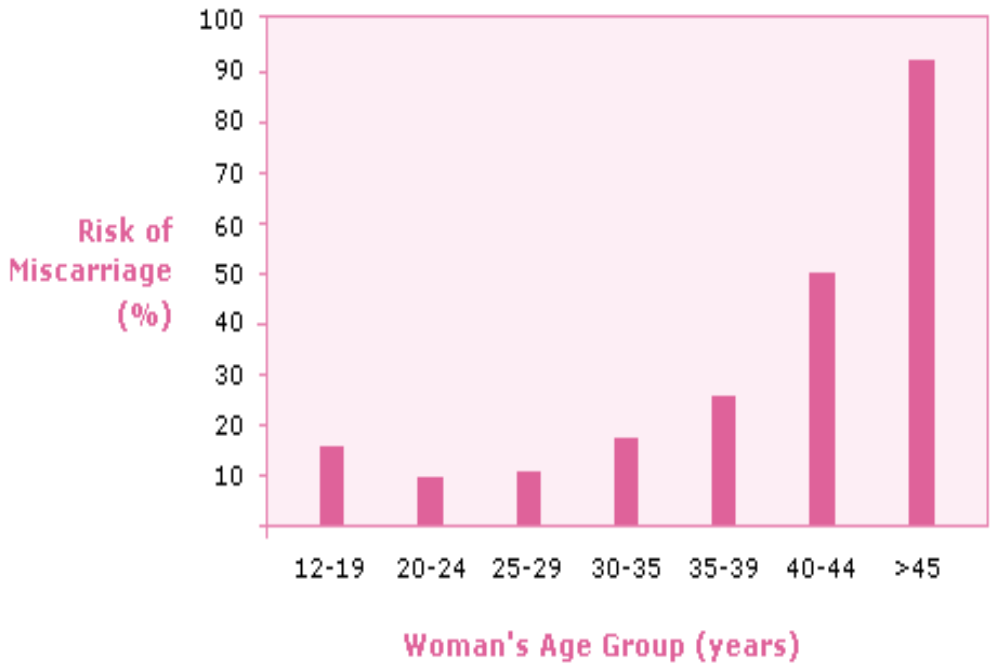

Figure-1: *Risk of miscarriage with maternal age (www.preconception.com.au/Images/Graphs/2d.gif)*