PCR GENETIC EXPRESSION OF INTERLEUKIN 37 IN THE EUTOPIC AND ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS

Thesis

Submitted for Partial Fulfillment of the MD Degree

in Gynecology and Obstetrics

By

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Above all and first of all; all thanks to **ALLAH** the source of all knowledge, by whose abundant aid this work has come to fruition.

It has been a great honor to proceed this work under the supervision of **PROF. IHAB FOUAD SERAG ELDIN ALLAM** Professor of Obstetrics and Gynecology Faculty of Medicine, Ain Shams University I am greatly indebted to him for suggesting and planning the subject, supervising the whole work, reading and criticizing the manuscript. I will never forget his unlimited help, continuous support, kind encouragement, constructive criticism and wise guidance. To him words of praise are not sufficient.

I would like also to express my sincere gratitude and appreciation to **PROF. AMGAD ALSAID ABOU – GAMRAH** Professor of Obstetrics and Gynecology Faculty of Medicine, Ain Shams University for his helpful guidance, valuable advice, meticulous care, great effort and generous help and support throughout this work.

I`m particularly very grateful to **DR. AHMED MOHAMED ABBAS** Lecturer of Obstetrics and Gynecology Faculty of Medicine, Ain Shams University for his time and advice for suggesting, reading and supervision throughout the practical part of this work.

I`m particularly very grateful to **DR. DINA ALY MOHAMED ALY RAGAB** Lecturer of Clinical pathology Faculty of Medicine, Ain Shams University for her time and advice for suggesting, reading and supervision throughout the practical part of this work.

Contents

List of Abbreviations

CA-125	Cancer Antigen-125.
CCL	Motif Chemokine Ligand.
CD	Cluster of differentiation.
CGH	Comparative genomic hybridization.
cIVF	Conventional Invirto Fertilization.
CXCL	Chemokine (C-X-C motif) ligand.
DCs	Dendritic cells.
DIE	Deep infiltrating endometriosis.
eEPs	Endometrial, epithelial progenitor cells.
eESCs	Ectopic endometrial stromal cells.
EFI	Endometriosis fertility index.
ELISA	Enzyme-linked immunosorbent assay.
eMSCs	Endometrial mesenchymal stem cells.
G-CSF	Granulocyte-colony stimulating factor.
GM-CSF	Granulocyte-macrophage colony stimulating factor.
GnRH	Gonadotropin-releasing hormone.
G-Rg3	Ginsenoside-Rg3.
G-Rh2	Ginsenoside Rh2.
IBD	Inflammatory bowel disease.
IL	Interleukin.
IVF	Invitro fertilization.
MCP-1	Monocyte chemoattractant protein-1.
MHC	Histocompatibility complex.
MIF	Migration inhibitory factor.
MSCs	Mesenchymal stem cells.
mTOR	Mammalian Target of Rapamycin.
PBMC	Peripheral blood mononuclear cell.

PI3K/AKT	Phosphoinositide 3 kinase/protain kinase B.
PPD	Protopanaxadiol.
PPT	Protopanaxatriol.
PTEN	Phosphatase and tensin homolog.
QOL	Quality of life.
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SHiP	Spontaneous haemoperitoneum in pregnancy.
SIGIRR	Single Immunoglobulin Domain-Containing IL1R-Related Protein.
siRNA	Small interfering RNA.
SMAD3	Mothers against decapentaplegic homolog 3.
SNPs	Single nucleotide polymorphisms.
STAT3	Signal transducer and activator of transcription 3.
TLR	Toll-like receptor.
TNF	Tumor necrosis factor.
VEGF	Vascular endothelial growth factor.
VEGFR2	Vascular endothelial growth factor receptor two.

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A protocol of thesis
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INTRODUCTION

Endometriosis is defined as the presence of normal endometrium abnormally implanted in locations other than the uterine cavity. Depending on the area identified, endometriosis is characterized as endopelvic or extrapelvic ***(Yu et al., 2013).*** The enterpelvic ectopic implants are located in the minor pelvis, the ovaries, the fallopian tubes and the uterosacral ligaments. Whereas, the more unusual extrapelvic implantation sites are the abdominal wall, scars of the perineum, the urinary and gastrointestinal tract and the nasal mucosa. Endometriosis can affect any woman from befor menarche until postmenopause ***(Simoglou et al., 2012).***

Endometriosis is the most common cause of chronic pelvic pain in females. Its prevalence has been estimated to 1-2% of reproductive age in females and it is more common (15-25%) among women with infertility problems **.**The prevalence is 40-60% among women with dysmenorrhea and it is extremely rare after menopause, because of the estrogen dependence of the ectopic tissue ***(Bulletti et al., 2010).*** The relapse of endometriosis during menopause has been correlated with hormonal replacement therapy ***(Giarenis et al., 2009).***

The etiology of the disease is complex, and is likely to result from a combined interplay of genetic and reproductive risk factors. Endometriosis has an estimated heritability of about 51% ***(Treloar et al., 1999).***

One of the theories is that peritoneal fluid (PF) in women with endometriosis is abundant in activated macrophages that secrete a variety of local products, such as growth factors and cytokines. ***(Harada et al., 2001; Sakamoto et al., 2003)***. Therefore, several growth factors, cytokines, immune cells and hormones in eutopic and ectopic endometrium, are seen as playing a role in the pathophysiology of endometriosis-related infertility ***(Nothnick, 2001).*** Abnormalities inherent to the eutopic endometrium that are not located in the endometrium of women without endometriosis are likely to be involved in the ectopic growth outside the uterine cavity ***(Ulukus et al., 2006 and Li et al., 2011).***

Cytokines are the main mediators and communicators of the immune system. Although these polypeptides are mostly produced by immune cells, most nucleated cells also produce cytokines, though in lesser quantities. Immune cells use cytokines to coordinate the host response to infection or trauma via autocrine and paracrine signaling. Based on their immune-regulatory role, cytokines are broadly classified as either pro- or anti-inflammatory. Proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) primarily initiate and amplify the inflammatory response ***(Cameron and Kelvin, 2003)***.

Anti-inflammatory cytokines such as IL-4, IL-6, and IL-10 primarily regulate the intensity and duration of the inflammatory response by suppressing the effects of proinflammatory cytokines, although some have inflammatory roles as well ***(Cameron and Kelvin, 2003)***.

The normal immune response to pathogens or injury entails a delicate balance of inflammatory and anti-inflammatory cytokines and regulators in order to be effective and remain safe for the host. Thus, cytokine dysregulation is recognized as an important aspect of the pathogenesis of numerous conditions, including endometriosis. Peritoneal fluid contains higher concentration of proinflammatory and angiogenic cytokines presumably produced from immune cells such as macrophages and from the lesion itself, which contribute to the pathogenesis of endometriosis ***(Jin et al., 2008).***

Interleukin 37 (previously called IL-1F7) is a discovered member of the IL-1 family. Interleukin 37 is located both in the cytoplasm and in the nucleus and is also secreted ***(Nold et al., 2010).*** The key role of IL-37 is inhibiting both innate and adaptive immunity by decreasing expression of pro-inflammatory cytokines ***(Bulau et al., 2014).*** Anti-inflammatory activity of IL-37 requires IL-18Ra and IL-1R8 to function together ***(Nold-Petry et al., 2015).*** A recent study demonstrates that IL-37 as an anti-inflammatory cytokine is increased in patients with various autoimmune and inflammatory diseases.

A previous study reported that the immunostaining score of IL-37 was significantly higher in the eutopic endometrium (4.79 ± 1.91) and ectopic endometrium (7.71 ± 1.78) of women with ovarian endometriosis compared to that of controls without endometriosis (3.27 ± 1.62) ***(Jiang et al., 2015).***

AIM OF THE STUDY

This work aims to study the PCR genetic expression of interleukin 37 in the eutopic and ectopic endometrium of women with endometriosis in comparison with controls.

HYPOTHESIS

In women with endometriosis, the PCR genetic expression of Interleukin 37 in the eutopic and ectopic endometrium may be highly expressed in comparison to normal ones.

RESEARCH QUESTION

In women with endometriosis is the PCR genetic expression of Interleukin 37 in the eutopic and ectopic endometrium highly expressed in comparison to that of controls?

PATIENTS AND METHODS

Setting:

The study will be conducted in Ain Shams University Maternity Hospital in laparoscopy department.

Study Design:

Case control study will be conducted in laparoscopy department in Ain Shams University Maternity Hospital to study the association between expression of interleukin 37 in the eutopic and ectopic endometrium of women with endometriosis in comparison with controls. The PCR genetic expression of interleukin 37 will be conducted in the clinical and chemical pathology department. The study will be performed form October 2016 to October 2018.

Sample Size Justification:

The required sample size has been calculated using the IBM© Sample Power© Software (IBM© Corp., Armonk, NY, USA).

The primary outcome measure is the PCR genetic expression of interleukin 37 (IL-37) in the eutopic and ectopic endometrium.

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So, it is estimated that a sample size of 23 patients with ovarian endometriosis and 23 controls would achieve a power of 80.1% (type II