



Effect of Letrozole on Surgically Induced Endometriosis in a Rat Model

Thesis

Submitted for Partial Fulfillment of the Master
Degree in Obstetric & Gynecology

By

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List of Abbreviations

AIs	: Aromatase inhibitors
COX	: Cyclooxygenase
EC	: Endothelial cells
EPCs	: Endothelial progenitor cells
FDA	: Food and Drug Administration
FSH	: Follicle stimulating hormone
GnRH	: Gonadotropin-releasing hormone
HIF	: Hypoxia-inducible factor
IHC	: Immunohistochemistry
IUI	: Intrauterine insemination
IVF	: In-vitro fertilization
NRP	: Neuropilins
OCP	: Oral Contraceptives
PCOS	: Polycystic ovary syndrome
PGE2	: Prostaglandin E2
PIGF	: Placental growth factor
ROS	: Reactive oxygen species
VEGF	: Vascular endothelial growth factor

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PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRIC & GYNECOLOGY

**Title of the Protocol: Effect of Letrozole on Surgically
Induced Endometriosis in a Rat Model**

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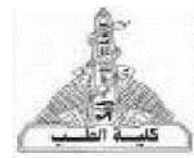
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2017



**What is already known on this subject? AND
What does this study add? (Maximum 6 lines) “References are not
needed**

Endometriosis is a chronic disease that requires a life-long management plan. Medical treatment of endometriosis depends on pelvic pain and presence of infertility. Medical treatment includes analgesics and hormonal therapy. Hormonal therapy includes combined oral contraceptive pills, progestogens, GnRH analogues and danazol. This research will study the effect of aromatase enzyme inhibitors as letrozole on endometriosis. Letrozole may be used for treatment of endometriosis.

**1. INTRODUCTION/ REVIEW (Maximum 1000 words)
“References are needed”**

Endometriosis is a chronic inflammatory condition in which foci of endometrial tissue grow outside of the uterine cavity. The presence of extra uterine endometrial tissue is associated with pelvic pain and infertility (**Giudice and Kao, 2004**). Endometriosis was estimated to affect 176 million women of childbearing potential all over the world (**Adamson and Pasta, 2010**).

Endometriosis is an estrogen dependent disease and estradiol supports growth and inflammation process in endometriotic lesions. Almost all treatments for endometriosis (GnRH agonists, progestins, danazol, OCP) have an effect on endometriosis by decreasing ovarian estrogen level or antagonising it (**Giudice, 2010**).

Ectopic endometrium expresses high levels of the aromatase P-450 (**Noble et al., 1996**). This enzyme is critical for estrogen (E) biosynthesis as it catalyzes the conversion of androstenedione(A) to estrone (E1) and testosterone (T) to estradiol (E2) (**Bulun et al., 2001**).

Prostaglandin E2 (PGE2) is the most potent inducer of aromatase expression and activity in endometriosis-derived stromal cells in vitro (**Noble et al., 1997**). In addition, it has been



shown that E2 induces Cyclooxygenase-2 (COX-2) expression and PGE2 production (*Tamura et al., 2004*). These mechanisms comprise a positive feedback cycle that favors the continuous local production of E2 and PGE2 in endometriotic lesions (*Attar and Bulun, 2006*).

In addition, it is known that both E2 and PGE2 induce the expression of vascular endothelial growth factor (VEGF) and, in turn, VEGF induces the expression of COX-2 (*Albrecht et al., 2003; tamura et al., 2006*). Vascular endothelial growth factor is one of the most important angiogenic factors involved in the pathogenesis of endometriosis (*Groothuis et al., 2005*). It has been observed that VEGF expression is increased in eutopic and ectopic endometrium from patients with endometriosis (*Bourlev et al., 2006; Takehara et al., 2004*).

Letrozole is a third-generation aromatase inhibitor, suppressing local and systematical estrogen production. Aromatase inhibitors are currently used as treatment for breast cancer (*Goss et al., 2003*). Other investigators have used aromatase inhibitors to treat cases of endometriosis (*Santen and Harvey, 1999; Mousa et al., 2007*).

2. AIM/ OBJECTIVES (Maximum 300 words)

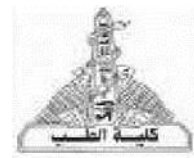
To evaluate the effect of letrozole on ectopic endometrial growth and on the expression of VEGF in rat model of surgically induced endometriosis.

Research Hypothesis

In rat model of surgically induced endometriosis, oral letrozole administration may suppress the growth of endometriosis.

Research question

In rat model of surgically induced endometriosis, does oral letrozole suppress the growth of endometriosis?



3. METHODOLOGY:

Patients and Methods/ Subjects and Methods/ Material and Methods (Maximum 1000 words)

Type of study

Experimental prospective study

Study setting

Medical research center, Ain Shams University Hospitals.

Study period

Seventy two days (72 days)

Study population

Non-pregnant, adult and sexually mature wistar albino female rats will be used to create a model for the experimental induction of endometriosis. they will be 90 days old and their weights will range from 150 to 200 gm.

Sample size

At the end of the research we need twenty rats to reach the final stage of the research and to be evaluated.

Etical consideration

All individuals who will be involved in animal research will receive explicit instructions on handling and caring with the experimental animals in compliance with the international guidelines for the care and use of experimental animals.

Study Intervention

Adult, non-pregnant Wistar albino female rats will be used to create a model for the experimental induction of endometriosis. The rats will be caged individually in a controlled environment with 12-h light/dark cycle. The animals will be sexually mature and would have demonstrated normal estrous cycle changes in vaginal smears. All rats will be left for 10 days prior to the experimental procedures to become adapted to the

experimental environment, during this period vaginal smear will be done and examined microscopically for determination of regularity of estrous cycle before the operation. Rats will be divided into two equal groups, letrozole and control group.

Study procedures

All animals will be anesthetized by intraperitoneal administration of Ketamine hydrochloride (80mg/kg BW) and Xylazine (5mg/kg BW). All the rats will undergo three consecutive surgical laparotomies.

1st laparotomy

Endometriosis will be induced surgically when using aseptic technique, a ventral midline incision about 5 cm long will be made to expose the reproductive organs. A distal segment 1cm in length will be resected from The left uterine horn, the endometrial layer will be separated and trimmed into a $5 \times 5 \text{ mm}^2$ piece. Next, the trimmed endometrial piece will be sutured to the inner side of the abdominal wall with 4-0 vicryl sutures. Finally the peritoneal cavity, the abdominal wall and the skin will be closed (*Korbel et al., 2010*).

1st Postoperative period

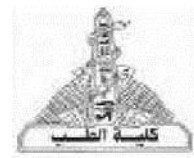
After the first endometriosis-inducing operation, all the rats will be observed for 30 days without any systemic medications. They will receive only topical antibiotics and topical analgesics for postoperative care.

2nd laparotomy

By the end of the 1st postoperative period, all rats will undergo a second exploratory laparotomy to observe the growth of the endometriotic implants. The surface sizes of the implants will be recorded (length in mm $\times$ width in mm). The endometriotic tissues will be photographed, then followed by the closure of the peritoneal cavity, abdominal wall and skin.

2nd postoperative period

All the animals will be allowed to rest for 7 days after the second laparotomy for postoperative care. Then we will



start to give medication.

Group 1 (Study group):

Rats will be given 0.2 mg/kg/day of oral letrozole (**Li et al., 2016**). Letrozole tablets (2.5 mg, Femara, Novartis, Switzerland) will be crushed and suspended in normal saline. The treatment period will last for 24 days.

Group 2 (Control group):

Rats will be given oral normal saline.

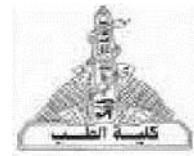
3rd laparotomy

By the end of the medication period, the rats will undergo the third laparotomy. The rats will be euthanized by Ketamine overdose anesthesia. After opening of the abdominal cavity, the endometriotic implants will be measured and photographed as previously described. Next, the implants will be excised and trimmed, a portion of each implant will be fixed in formalin 10% and then embedded in paraffin for histopathological examination and immunohistochemistry.

Histopathological examination and immunohistochemistry

The implant specimens will be sliced at 4- μ m thickness. Hematoxylin and eosin (H-E) staining will be used to evaluate the growth of the endometriotic tissues. In the immunohistochemistry (IHC) procedure, the slides will be first deparaffinized and rehydrated, followed by incubation in a 3% H₂O₂ solution to deactivate endogenous peroxidase. After using normal goat serum to block nonspecific antigens, the slides will be incubated with primary antibodies overnight at 4 °C. The primary antibodies will include rabbit anti-rat VEGF polyclonal antibodies (**1:100; Abcam, Cambridge, UK**). Next, the slides will be incubated with the secondary antibodies, followed by counterstaining with hematoxylin (**ABC kit; Abcam, Cambridge, UK**). For immunohistochemistry analysis, the pathologist evaluating the slides will be blinded to all the groups.

The percentage of positive stained cells will be scored from 0 to



3 as follows; 0: less than 5%, 1: 5%– 25%, 2: 26%–50%, and 3: more than 50%.

The staining intensity of the cells will be scored as follows:

0: negative, 1: weakly positive, 2: moderately positive, and 3: strongly positive.

The IHC score for each rat will be calculated by multiplying the two scores (staining intensity and percentage of positive stained cells).

Enzyme-linked immunosorbent assay

The serum levels of E2 will be assayed three times using ELISA kits by taking blood sample from retroorbital venous plexus.

1st blood sample will be taken before the 1st laparotomy

2nd blood sample will be taken before starting treatment by one day

3rd blood sample will be taken at the end of the study just before 3rd laparotomy

Statistical analysis

Statistical analysis will be performed by IMB SPSS statistics version. Data will be given as a median (min-max). Man Whitney U test will be used to compare the differences between treatment and control groups and Wilcoxon signed rank test will be used to compare the pre and post treatment differences within groups. P value less than or equal 0.05 will be considered a statistically significant.

Outcome measures

Primary out come

Immuno reactivity score of VEGF

Secondary outcome

Histopathological examination

Implant surface size

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