Increased Expression of Matrix Metalloproteinase-9 in the Ectopic Endometrial Tissue of Women with Endometriosis

Prospective study

Thesis

Submitted for the partial fulfillment of the Master degree in Obstetrics and Gynecology.

By

Mohamed Abd El-Mordy Said El-Sannan

(M.B., B. Ch.)
Faculty of Medicine
Menoufia University
2009
Resident of Obstetrics and Gynecology
In Shebin Elkom Teaching Hospital

Under supervision of

Dr. Noha Hamed Rabei

Assistant Professor of Obstetrics & Gynecology

Faculty of Medicine Ain Shams University

Dr. Mohammed Hussain Mostafa

Assistant Professor of Obstetrics & Gynecology

Faculty of Medicine Ain Shams University

Dr. Ragea Ahmed Fahmy

Assistant Professor of Pathology

Faculty of Medicine Ain Shams University

Ain Shams University

2016

Dedication

My deepest gratitude to my beloved family, my father, my mother, my wife and my sons for their Obsessive love and support throughout my life.

Acknowledgement

It is a great honor to express many deep gratitude and appreciation to ***Dr.*** **Noha Hamed Rabei,** Assistant Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, for her encouragement, creative suggestions, and whole hearted support, both scientific and moral and her guidance throughout the study.

I wish to express my sincere thanks to ***Dr.*** **Mohammed Hussain Mostafa** Assistant Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, for his continuous supervision, sincere assistance and continuous support during all stages of thesis, unlimited valuable advice and critical reviewing of the manuscript are greatly admitted.

I would like to express my deep appreciation and sincere gratitude and express my sincere gratefulness, to ***Dr.*** **Ragea Ahmed Fahmy,** Assistant Professor of Pathology, Faculty of Medicine ,Ain Shams University, for here precious assistance and invaluable practical guidance. The successful completion of this work was the result of her continuous support and fruitful discussions.

Last but not least I praise and express my utter and wholehearted thanks to ***Dr. Mohamed Hussein Mostafa,*** Assistant Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University for his overwhelming help, ingenious supervision, continuous guidance and valuable comments were essential in the completion of this thesis.

Mohamed Abd El-Mordy Said El-Sannan

List of contents

List of Abbreviations

AFS	American Fertility Society
ARDS	Acute respiratory distress syndrome
CA-125	Carcinogenic embryonic antigen 125
COCPs	Combined oral contraceptive pills
DIE	Deep infiltrating endometriosis
ECM	Extracellular matrix
EIF	Endometriosis inducing factor
ELISA	Enzyme linked immunosorbent assay
ET	Embryo transfer
GnRH	Gonadotropin-releasing hormone
IFN	Interferon
IL	Interleukin
IVF	In vitro fertilization
LUNA	Laparoscopic uterine nerve ablation
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MRNA	Messenger Ribonucleic acid
MSCs	Mesenchymal stem cells
NSAIDs	Non-steroidal anti-inflammatory drugs

PCNA	Proliferating cell nuclear antigen
PGE2	Prostaglandin E2
PID	Pelvic inflammatory disease
PMNs	Polymorph nuclear neutrophils
TIMP	Tissue inhibitor of metalloproteinase
TNFα	Tumor necrosis factor
TVS	Transvaginal ultrasound
VEGF-A	Vascular endothelial growth factor A
ZN2+	Zinc

List of Figures

List of Tables

Increased Expression of Matrix Metalloproteinase-9 in the Ectopic Endometrial Tissue of Women with Endometriosis

Prospective study

Thesis

Submitted for the partial fulfillment of the Master degree in Obstetrics and Gynecology.

By

Mohamed Abd El-Mordy Said El-Sannan

(M.B., B. Ch.)
Faculty of Medicine
Menoufia University
2009
Resident of Obstetrics and Gynecology
in Shebin Elkom Teaching Hospital

Under supervision of

Dr. Noha Hamed Rabei

Assistant Professor of Obstetrics & Gynecology

Faculty of Medicine Ain Shams University

Dr. Mohammed Hussain Mostafa

Lecturer of Obstetrics & Gynecology

Faculty of Medicine Ain Shams University

Dr. Ragea Ahmed Fahmy

Assistant Professor of Pathology

Faculty of Medicine Ain Shams University

Ain Shams University

2014

INTRODUCTION

Endometriosis is a common gynecological disorder affecting about ten to fifteen percent of women in the reproductive age and up to 50% of infertile patients are affected by endometriosis. The disease is characterized by the ectopic location of endometrial cells in tissues other than the uterine cavity its main symptoms are chronic pelvic pain, dyspareunia, and infertility **(Goldman and Cramer, 1990**).

Although the association between endometriosis and infertility has led to extensive research efforts, the origin of endometriotic cells remains elusive.The most widely accepted theory to explain peritoneal endometriosis is retrograde menstruation **(Brosens and Brosens, 2000).**

According to this theory, the disease arises from ectopic implantation and growth of endometrial tissue that reaches the peritoneal cavity by tubal reflux **(Brosens and Brosens, 2000).**

However, besides the fact that this phenomenon has also been described in healthy women and can thus be viewed as a physiological process **(Halme et al., 1984)**, several lines of evidence indicate that endometriotic lesions are biologically different from normal uterine endometrium **(Kressin et al., 2001).**

In order for ectopic implantation and growth to occur, endometrial tissue must first attach itself to the host tissue, invade it and to locally destruct the extracellular matrix (ECM), then obtain from the local vasculature its own blood supply **(Giudice et al., 1998)**.

Degradation of the extracellular matrix (ECM) is therefore a basic step in the formation of new vessels in angiogenesis with regard to tissue remodeling (**Yasunaga et al., 1989).**

Many factors are important for the degradation of ECM and the implantation of endometrial tissue in ectopic sites**,** notably cathepsin D **(Suzumori et al., 2001)**, plasminogen **(Sillem et al 2001)** and matrix metalloproteinases (MMPs) **(Kokorine et al., 1997).**

One of the most prominent phenotypic features of endometriotic lesions is the expression of specific matrix metalloproteinases (MMP). Constituting a group of matrix-degrading zinc enzymes, MMP are not only known to play a pivotal role in the initiation of menstrual bleeding, but have also been shown to contribute to implantation and further invasion of seeded endometriotic explants **(Singer et al., 1997; Henriet et al., 2002).**

MMPs form a multigenic family of proteolytic enzymes that depend on zinc for activation **(Van Wart and Birkedal-Hansen, 1990).**They are first secreted in their latent form as proenzymes and can be activated later **(Salamonsen et al., 1997).**

MMPs have different specialties, even if there are considerable overlaps, and together they are able to break down most ECM components, including the different types of collagens that make up the basement membrane (**Freitas et al., 1999).**

The ECM degradation, operated by the MMPs, is closely regulated by tissue inhibitors of metalloproteinases (TIMPs) under normal physiological conditions such as tissue repair **(Okada et al., 1987),** embryogenesis **(Pajouh et al., 1991)** and menstruation **(Osteen et al., 1994)**. Imbalance between MMP and TIMP expression has been involved in various medical conditions.

They **have** detected higher levels of MMP-2 and -9 expressions in eutopic and in ectopic endometria of patients with endometriosis than in corresponding healthy controls. **(Chung et al. (2001, 2002).**

Several recent studies highlighted the role of MMPs in endometriosis. **(Koks et al. 2000)** reported that MMP activity in menstrual serum is different from and more intense than MMP activity in peritoneal fluid, and that these enzymes may be involved in the early invasion of menstrual endometrium into the ECM of the peritoneum

Different MMPs, such as MMP-1 **(Kokorine et al., 1997)**, MMP-2 **(Wenzl and Heinzl, 1998)**, MMP-7 **(Bruner-Tran et al., 2002)** and MMP-9 **(Liu et al., 2002)**, were reported to have increased expression in endometriotic lesions.

Matrix metalloproteinase -9 increases in many conditions such as breast cancer, ovarian carcinoma, endometriosis, osteoarthritis, myocardial infarction and rheumatoid arthritis.

Matrix metalloproteinase -9 is affected by many factors as prostaglandin (PGE2), tumor necrosis factor-α (TNF-α), Interleukin (IL- 1β), interferon-γ (IFN-γ) and tissue inhibitors of matrix metalloproteinase (TIMP).

Aim of the work

The aim of the present study is to evaluate MMP-9 forms in the ectopic and eutopic endometrial tissue of women with endometriosis.

Research question

What is the value of Matrix Metalloproteinase-9(MMPs-9) as a diagnostic marker in endometriosis?

Hypothesis

MMPs-9 can be used as a marker for early diagnosis of endometriosis.

Subject and Method

1-Type of study:

Prospective study.

2- Site of the study:

Ain Shams Maternity University Hospital.

3-Duration of the study:

From May 2014 to septemper 2015

4- Study population:

Size: As the accuracy of the marker (MMPs-9) was 70% and at a power 80% and 95% CI.

The estimated sample will be 28 cases and 28 as control (EPI_INFO version 6)

Sample size justification

Based on the study performed by **Bayramoglu et al., 2005:**

is used for calculation of sample size guided by the following data:

> Power of the test = 80%

>confident level =95%

> Type 1 error &error =5%

5-Inclusion Criteria:

Cases:

1. Patients aged between 20 and 45 years.
2. Patients suffering from endometriosis.
3. Endometriosis will be identified during

- Laparoscopy in women consulting for infertility.
- Open surgical procedures for severe endometriosis.
- Hysterectomy for non-malignant lesions such as fibroids.

Control:

1. Aged between 20 and 45 years.
2. Patients are fertile and do laparoscopy for other causes such as:

- Ovarian cystectomy.
- Ovarian drilling in cases of polycystic ovary.
- Ectopic pregnancy.

3. There is no evidence of endometriosis upon laparoscopy.

4. Patients suffering from vaginal bleeding.

6- Exclusion criteria:

1. Patients with a history of pelvic inflammatory disease or malignancy, adenomyosis uteri.

2. Patients exposed to steroids or hormonal medication (GnRH) within the last 6 months prior to surgery.

7- Methodology:

All patients will be subjected to the following:

1- Consent.

2- Detailed history will be recorded: as regards age, sex, residence, socioeconomic status, onset, progression, and treatment.

3- Full general examination.

4- Full clinical examination including chest, abdomen, cardiac and pelvic examination.

5-Investigations: Laboratory investigations including:

I. CBC

II. Random blood sugar

6- Conventional ultrasound examination to determine any pelvic lesions and adhesions.

7- Ectopic endometriotic tissues will obtained during laparoscopic or open surgical procedures for severe endometriosis or during hysterectomy for non-malignant lesions such as fibroids.