



## The effect of ENEAS application in patients with endometriosis and its influence on the level of IL-8 and MCP-1

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### ABSTRACT

Endometriosis is a gynecological disease that endometrial cells develop outside the uterus. This event happens when the endometrial glands grow outside the endometrium and uterine muscles, especially in the pelvis. Although endometriosis is widespread, the clinical manifestations of the disease are very different, and it is challenging to adapt to the conventional classification system to divide patients into homogeneous groups. Given the importance of endometriosis, a correct, accurate, and timely diagnosis of this disease can significantly prevent its complications. Using health-related software is one of these ways. Enhanced Endometriosis Archiving Software (ENEAS) is a web-based application based on one of the most widely used open-source database management systems (MySQL), allowing the direct link to other open-source software for data management and storage. In the current study, the effect of ENEAS application was considered in patients with endometriosis, and its influence on IL-8 and MCP-1 gene expression was evaluated. For this purpose, 100 women with endometriosis were divided into two groups of 50 patients. The first group (control group) was examined by a gynecologist and received medication and treatment. In the case group, their demographic and clinical information were entered into ENEAS software. To study the expression of the IL-8 gene and MCP-1 gene, after collecting 5 ml of blood samples in tubes containing anticoagulant, RNA extraction was performed by Total RNA Purification Kit (Cat. 17200, 37500, 17250). Then cDNA synthesis was performed for this purpose, and a Bioneer DNA synthesis kit (South Korea) was used. The results showed that the expression level of the IL-8 gene in the case group was significantly reduced compared to the control group ( $P = 0.035$ ). MCP-1 gene expression was also decreased compared to the control group, but this decrease was not significant. Therefore, those who used this application for treatment had reduced expression of IL-8 and MCP-1 genes. This event indicates that this application has reduced the amount of inflammation caused by endometriosis with proper analysis.

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### Introduction

Endometriosis is a gynecological disease characterized by the presence and growth of endometrial glandular tissue and stroma outside its original location (uterine cavity) (1). Endometrial tissue deposition is most common in the pelvis (ovaries, peritoneum, uterosacral ligament, Douglas space, rectovaginal septum) (2). Endometriosis is a common disease of reproductive age; it is found in 10% of fertile women and 50-20% of infertile women (1). This common disorder is a chronic inflammatory disease associated with a general inflammatory response in the peritoneal cavity and it is called a disease with an immunological background (3). The etiological immune/inflammatory cause of endometriosis is related to increased concentrations of activated macrophages, cytokines, B and T cells,

growth factors, and angiogenesis stimulators. Angiogenesis is essential pathophysiology in the development of endometriosis (4).

Vascular endothelial growth factor (VEGF) is a critical mediator in angiogenesis and has strong potential in physiological and pathological angiogenesis. This disorder is one of the most well-known conditions for changes in angiogenesis (5). The expression of many other angiogenic growth factors and cytokines (FGF, IGF, PGF, IL-1, and IL-8) has also increased in the endometrial tissue of women with endometriosis (6-8). Monocyte chemoattractant protein-1 (MCP1) is produced by monocytes, endothelial cells, fibroblasts, and T cells increasing the migration of monocytes from the bloodstream to tissue and converting them to macrophages (8). It eliminates infection from the body

by inducing phagocytosis. MCP1 is a member of the cytokine family and plays a vital role in the secretion, release, and function of monocytes, macrophages, neutrophils, and lymphocytes (9). This cytokine is involved in the immune response to infection and inflammation and has antitoxic effects following the administration of drugs. It has different functions depending on the target organ, the dose of the medicine, the role of other cytokines, and drug administration. For example, MCP stimulant medicines are used in the lungs, while MCP blocker medicines are used in the kidney and endometriosis disease (10, 11).

The most proper diagnostic and treatment methods have not yet been identified for endometriosis, and the management of this chronic disease is complex in most cases (2, 12). For endometriosis research, high costs and expertise are needed to collect reliable and detailed clinical history, imaging data, and the processing of tissue specimens (12). Such information is of the most incredible value when collected using systematic protocols and accompanied by detailed clinical classification/cataloging of patients (13). Therefore, developing a global registry of endometriosis bio-banks and databases is one of the main priorities for endometriosis research. For this purpose, Enhanced Endometriosis Archiving Software (ENEAS) has been developed as the first databank to store, retrieve, compare, and correlate data collected on the management of patients diagnosed or suspected endometriosis in different centers (14).

Based on the importance of health-related software (14), this study investigated the effect of ENEAS application on the expression of IL-8 and MCP-1 genes in patients with endometriosis.

## Materials and methods

### Studied patients

In this case-control study, 100 women with endometriosis were divided into two groups of 50 patients. The first group (control group) was examined by a gynecologist and received medication and treatment. In the case group, their demographic and clinical information were entered into ENEAS software. According to the database prepared in this software, the type and amount of medicines were determined, and after approval by a gynecologist, they were treated. Two months after treatment, blood

samples were taken from these individuals for further evaluation.

### Genetic evaluations

To study the expression of IL-8 and MCP-1 gene, after collecting 5 ml of blood samples in tubes containing anticoagulant, RNA extraction was performed by Total RNA Purification Kit (Cat. 17200, 37500, 17250), and then cDNA synthesis was performed for this purpose and Bioneer DNA synthesis kit (South Korea) was used. All steps were performed on ice and under the Laminar flow cabinet and sterile conditions. After RNA extraction and cDNA synthesis, the PCR reaction was performed in a volume of 10 $\mu$ l. 5 $\mu$ l of Mastermix 2X, 3 $\mu$ l of DEPC, 1 $\mu$ l of cDNA, and 0.5 $\mu$ l of primer were added to each micro-tube. Allele ID software was used to design the primers, and they were synthesized by BioTech (USA). The Beta-actin gene was used as a housekeeping gene to normalize the data. The sequence of primers is given in Table 1. The PCR program is listed in Table 2. DNAs were amplified by Comfort Thermocycler (Eppendorf, Germany).

**Table 1.** The Sequence of the primers designed with Allele ID software; gene (A), primer sequence (B), and annealing temperature (C)

A		B	C
IL-8	Forward	5'-TCTCTTGGCAGCCTTCCT-3'	52°C
	Reverse	5'-ACTGAACCTGACCGTACATGT-3'	
MCP-1	Forward	5'-CTGCTTGCTGCCATTCTC-3'	58°C
	Reverse	5'-CACACCTGCCTTTACACC-3'	
$\beta$ -Actin	Forward	5'-CCTCGCTCTCCACCTTCC-3'	61°C
	Reverse	5'-TGCCAATCTCGTCTCGTTTC-3'	

**Table 2.** Time and temperature of the real-time PCR cycles

Steps	Time	Temperature	Cycles
Primary			
Denaturation	10 minutes	95°C	1
Denaturation	15 seconds	95°C	35
Annealing	30 seconds	52°C-61°C	35
Extension	30 seconds	72°C	35

### Statistical analysis

For statistical analysis of data, SPSS software version 18 was used. Paired t-test was performed on the expression of MCP-1, IL-8, and beta-actin genes in both groups. *P*-value less than 0.05 were considered significant.

## Results and discussion

### Demographic and clinical evaluations

The mean and standard deviation of women age in the case group ( $33.72 \pm 0.61$  years) had a significant difference compared to the control group ( $34.85 \pm 0.71$  years) ( $P = 0.025$ ) (Table 3). The mean and standard deviation of body mass index of the case and control women were  $24.78 \pm 0.23$  kg/m<sup>2</sup> and  $19.33 \pm 0.42$  kg/m<sup>2</sup> ( $P = 0.048$ ), respectively. The frequency of infertility in the case group was almost equal to the control group so that 36% of the control group and 34% of the treatment group had infertility ( $P = 0.699$ ). Painful menstruation was not significantly different between the two groups.

**Table 3.** Demographic and clinical evaluations in control group and case group

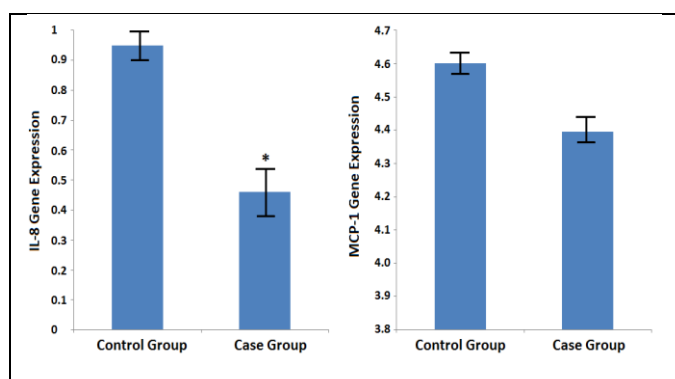
Variable	Control Group (n=50)	Case Group (n=50)	P-value
Age (year)	$34.85 \pm 0.71$	$33.72 \pm 0.61$	0.025
BMI (kg/m <sup>2</sup> )	$19.33 \pm 0.42$	$24.78 \pm 0.23$	0.048
Marital Status			
Married	43 (86%)	45 (90%)	0.421
Single	7 (14%)	5 (10%)	0.447
Infertility	17 (34%)	18 (36%)	0.699
Regular Menstruation			
Having	28 (56%)	26 (52%)	0.112
Not Having	22 (44%)	24 (48%)	0.098
Duration of menstrual bleeding			
≤4 days	11 (22%)	12 (24%)	0.672
5 days	25 (50%)	24 (48%)	0.721
≥6 days	14 (28%)	14 (28%)	1
Menstrual Pain			
Having	35 (70%)	33 (66%)	0.72
Not Having	15 (30%)	17 (34%)	0.78
premenstrual spotting ≥2 days	31 (62%)	32 (64%)	0.901
Family History			
Having	41 (82%)	37 (74%)	0.077
Not Having	9 (18%)	13 (26%)	0.061

### Genetic evaluations

This part showed that the expression level of the IL-8 gene in the case group was significantly reduced compared to the control group ( $P = 0.035$ ) (Figure 1). MCP-1 gene expression was also decreased compared to the control group, but this decrease was not significant.

Endometriosis refers to the growth of endometrial tissue outside the uterine cavity, which can occur inside and outside the pelvis (1). Endometriosis is most common in the pelvis, ovaries, posterior clivicle, uterine ligaments, pelvic peritoneum, and rectovaginal septum (15). When endometriosis is found in other body parts, such as the cervix, vulva, vagina, intestines, urinary system, abdominal wall, chest, lungs, and central nervous system, ectopic endometriosis will occur. Although many patients with endometriosis have no symptoms, they may be associated with menstrual cramps, intercourse pain, and chronic pelvic pain (16). Endometriosis is also seen in 30 to 50% of infertile women, which was similar to the results of our study. Causes of endometriosis include infertility, family history of endometriosis, obstruction of the normal outflow of menstrual blood from the body, history of pelvic infection, and uterine disorders (8). Accurate and reliable results of the leading causes of the disease are not available. Still, according to researches, in addition to environmental factors, hereditary factors also play an essential role in endometriosis in people with first or second-degree relatives. The risk of endometriosis is significantly increased in them (2). The results of our study also showed that 82% of the control group and 72% of the case group had a family history.

In the study by Burghaus *et al.* (17), which was performed as a case-control study and by using logistic regression model, the age of menarche onset, menstrual period, menstrual cycle length, number of pregnancies, ectopic pregnancies, and smoking were considered as risk factors. In line with the results of our study, which showed that 62% of the control group and 64% of the case group had premenstrual spotting, Hardiman *et al.* (18) showed that premenstrual spotting for two days or more was associated with endometriosis, which is identified as a better predictor than painful menstruation and painful



**Figure 1.** The relative IL-8 gene and MCP-1 gene Expression in control group and case group

proximity. A study by Potischman *et al.* (19) found that high estrogen levels in the body significantly increased the risk of developing endometriosis. They also noted that estrogen levels were not associated with other factors in the occurrence of endometriosis and were an independent factor.

Given the importance of endometriosis, a correct, accurate, and timely diagnosis of this disease can significantly prevent its complications using health-related software is one of these ways (20). Enhanced Endometriosis Archiving Software (ENEAS) is a web-based application based on one of the most widely used open-source database management systems (MySQL), allowing the direct link to other open-source software for data management and storage (14). ENEAS can be installed locally on the server, which provides access to the database and any related functions through any computer connected to the local network (14). It can be used in a single hub or the Cloud without a local hardware load, allowing unacknowledged data to be shared in real-time and quickly used for research. Each patient has a personal form completed at the initial consultation within ENEAS and includes all relevant information, including name, nationality and date of birth, address, telephone number, and email address. Some of these are mandatory for further data entry. The classic website organization is chosen for the database collection, with a navigation bar on the left that allows the operator to move between macro events and their subsets quickly. The navigation bar includes clinical history, hospitalization, gynecology, outpatient counseling, and questionnaire. According to this application, patient data is stored, and comparing this data with the data of thousands of patients with endometriosis gives an overview of the disease to the obstetrician and the type and amount of medication easily obtained through this application (14). Evaluation of the performance of this application showed that the expression level of the IL-8 gene was significantly reduced. MCP-1 gene expression also decreased, but this decrease was not significant.

Interleukin 8 (IL-8) is a factor secreted by T lymphocytes that precisely differentiates B lymphocytes into plasma cells (producing antibodies) and is produced and secreted by hematopoietic and non-hematopoietic cells (21). Immunohistochemical studies on the human ovary have shown that IL-8 is

secreted from the corpus luteum (22). IL-8 causes folliculogenesis and steroidogenesis in granulosa cells. IL-8 also promotes new angiogenesis in the human ovary by increasing the production and secretion of VEGF (23). Therefore, its excessive increase can cause the pathological complication of ovarian hyperstimulation syndrome. It has also been reported that the concentration of IL-8 increases in the follicular fluid of infertile women with endometriosis. Therefore, this cytokine can play a role in the pathogenesis of infertility due to endometriosis (24, 25).

The results of this study showed that the use of ENEAS software enables gynecologists to compare the information of one patient to thousands of endometriosis patients in the software database, which can provide more accurate information for better treatment.

## Conclusions

Endometriosis is a condition in which cells similar to endometrial cells develop outside the uterus. This event happens when the endometrial glands grow outside the endometrium and uterine muscles, especially in the pelvis. Although endometriosis is widespread, the clinical manifestations of the disease are very different, and it is challenging to adapt to the conventional classification system to divide patients into homogeneous groups. In addition, the lack of standardization of surgery and postoperative treatment poses a challenge for accurate comparison of data. It increases the number of possible subtypes related to the initial preoperative diagnosis. Therefore, developing a global registry of endometriosis biobanks and databases is one of the main priorities for endometriosis research. For this purpose, Enhanced Endometriosis Archiving Software (ENEAS) has been developed as the first databank to store, retrieve, compare, and correlate data collected on the management of patients diagnosed or suspected endometriosis in different centers. In the current study, the effect of ENEAS application was considered in patients with endometriosis, and its influence on the level of IL-8 and MCP-1 gene expression was evaluated. The results showed that those who used this application for treatment had decreased expression of IL-8 and MCP-1 genes. This event indicates that this application has reduced the



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