

# Is HE4 a useful endometrioma marker?

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## Summary

**Purpose of investigation:** By the comparison between most used tumor marker trend (cancer antigen 125: CA 125 and human epididymal secretory protein E4: HE4) before and after laparoscopic surgery, the aim of the present study was to assess HE4 usefulness in ovarian benign cyst and endometrioma diagnosis. **Materials and Methods:** Thirty-eight patients were enrolled in this prospective study: 25 women underwent unilateral endometriosis ovarian cyst excision, 13 underwent benign ovarian cyst incision, and 26 were healthy controls. CA 125 and HE4 serum levels were estimated before surgery (in the early proliferative phase of the cycle) and one month after surgery. **Results:** A statistically significant decrease of CA 125 serum level was found after an endometrioma surgical excision but no decreases in HE4 serum level. **Conclusion:** In patients with endometrioma, no alteration was found in HE4 serum levels before and after surgery, while CA125 serum levels decreased after surgery. HE4 may better distinguish a malign cyst from benign one, but it is not useful in the diagnosis of low risk endometrioma.

**Key words:** HE4; Ca125; Ovarian cyst; Endometrioma.

## Introduction

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterus and it affects 3% to 10% fertile women [1]. Thirty-five percent of benign cysts needing surgery are endometriomas [2]. The gold standard for their diagnosis is laparoscopic inspection and histological confirmation [3]. Even if endometriosis cannot seem a premalignant condition, epidemiologic, histopathological and molecular data suggest that an endometriosis environment is associated to certain subtypes of ovarian cancer, such as endometrioid and clear-cell carcinomas [4, 5]. Several molecules, especially cancer antigen 125 (CA 125), have been studied for diagnosis of endometriosis, but no specific serum marker is available yet [6].

Elevated serum levels of CA 125 may be associated with a high false positive rate to benign gynaecological condition, such as endometriosis [7], and it distinguishes with a low sensitivity benign gynaecological condition from early-stage ovarian cancer [8].

Human epididymal secretory protein E4 (HE4) is a new tumor marker, more sensitive than CA 125 in the diagnosis of early ovarian cancer, especially in young women [9]. There are no evidences in literature regarding the use of HE4 in benign pelvic mass diagnosis but it could distinguish early stages of malignant ovarian cancer from endometriomas [10] and other benign adnexal masses.

The aim of the present study was to assess if tumor marker levels trend (HE4 and CA 125) varies in different forms of ovarian cysts (and among them, especially endometrioma) by their evaluation before and after laparoscopic removal.

## Materials and Methods

This prospective study was conducted in the period between October 2010 and June 2012 in the Center of Mini-Invasive Pelvic Surgery, Department of Health of Woman and Child, University of Padua.

Patients were divided in three groups: the first one, composed of women affected by symptomatic unilateral endometriomas, the second one, of those affected by unilateral ovarian cyst (cystic teratomas, serous, and mucinous cystadenomas) undergoing surgical laparoscopic treatment, and the third (controls, young healthy women) that did not receive surgery. Clinical symptoms such as dysmenorrhea, dyspareunia, chronic pelvic pain, and infertility clinical history were recorded and pelvic examination was preoperatively performed.

The inclusion criteria of this study were the following: 1) age > 18 and < 38 years; 2) normal ovulatory cycles with a duration between 26 and 35 days; 3) unilateral ovarian cyst with size more than three cm; 4) absence of endocrine disease (thyroid disease, diabetes mellitus, hyperprolactinemia, polycystic ovarian syndrome); 5) no prior ovarian surgery; 6) consent to participate in the study.

The exclusion criteria were: 1) malignant cysts; 2) E/P in place or in the six month prior to surgery; 3) therapy or a history of GnRH; 4) history of chemotherapy or radiotherapy; 5) pelvic inflammatory disease.

Informed consent was obtained from all patients and the study was approved by the present department's ethical committee (n.2735P).

Preoperative workup consisted in Pap smear, urinary and blood analysis including haemachrome, PT, PTT, and electrocardiography. Blood samples of surgical patients were collected in two periods: one month before surgery, in the early proliferative phase of the cycle, and one month after surgery (during the first menstrual cycle). Controls blood samples in the early proliferative phase of the cycle were also gained. Blood was picked up within 15-30 minutes in citrated vacuoneters and stored at 4°C. Serum samples aliquots were centrifuged with the speed of 4,000 rpm/sec at the temperature of 4°C with the aim to separate debris and cellular contents. They were frozen at -80°C.

Revised manuscript accepted for publication August 27, 2013

CA 125 and HE4 serum levels were measured with a method of chemiluminescence, the so-called Architect CA 125, HE4. The range concentration for CA 125 was 0-35 KU/L while for HE4 in premenopausal women it was < 70 pmol/L, according to manufacturer's instructions. All assays were tested in duplicates.

#### Surgical procedure

The procedure were executed under general anaesthesia by the same surgeon in day hospital as described below: laparoscopic pneumoperitoneum was obtained by CO<sub>2</sub> insufflation with an umbilical ten-mm trocar. Two five-mm ancillary trocars were introduced under direct laparoscopic observation. The first step was the exploration of the abdominal cavity to exclude endometriosis located out from the ovary. The removal of the cystic wall from the ovarian cortex was performed using scissors and grasping forceps. After identification of the cleavage plane, the stripping of the cyst was performed using two non-traumatic grasping forceps by cautious traction. All the procedures were performed without the spillage of the cyst's content. An intracortical suture was performed with a PDS 2.0 monofilament wire, approaching the ovary edge and not damaging the ovarian tissue. Bipolar forceps were never used to minimize the possible injury to the ovarian tissue. The ovarian cysts were removed using a disposable endobag.

Stated that elective laparoscopic surgery was performed for benign gynecologic conditions, antibiotic prophylaxis was not administered [11]. Every specimen was examined and the nature of the lesion was histologically confirmed. The patients were discharged on the same day or the day after surgery.

Statistical analysis was performed on the collected data using Statistics Package for Social Sciences software (SPSS 19.0) and  $p < 0.05$  was accepted as significance level. Pre- and post-operative CA 125 and HE4 serum levels were compared using Kruskal-Wallis One Way Analysis of Variance on Ranks and were reported as median values (range). Dunn's method for multiple comparison procedures was utilized.

## Results

Thirty-eight patients fulfilled the inclusion criteria of the study: 25 of them underwent surgery for endometrioma, 13 of them had surgically removed a benign ovarian mass (cystic teratomas, serous, and mucinous cystadenomas) and 26 women were recruited as controls. Their clinical characteristics are described in Table 1. The mean age of the patients was  $31.9 \pm 5.7$ , mean BMI was  $22.1 \pm 4.8$  kg/m<sup>2</sup>, mean cyst size was  $5.0 \pm 1.7$  cm.

Table 1. — Demographic characteristics of patients.

|                                  | Cysts            | Controls       |
|----------------------------------|------------------|----------------|
| Number of patients (n)           | 38               | 26             |
| Age (years)                      | $31.9 \pm 5.7$   | $29.8 \pm 5.2$ |
| BMI (kg/m <sup>2</sup> )         | $22.1 \pm 4.8$   | $21.5 \pm 2.2$ |
| Parity (n)                       | $0.3 \pm 0.5$    | $0.4 \pm 0.6$  |
| Size of ovarian cyst (cm)        | $5.0 \pm 1.7$    |                |
| Cyst volume (cm <sup>3</sup> )   | $92.0 \pm 120.0$ |                |
| Cyst site (n)                    |                  |                |
| right                            | 17 (44%)         |                |
| left                             | 21 (55%)         |                |
| Duration of surgery (min)        | $66.0 \pm 32.8$  |                |
| Intraoperative complications (n) | 0                |                |
| Blood loss (cc)                  | $24 \pm 22$      |                |

No intraoperative complications were reported. Mean blood loss was  $24 \pm 22$  cc.

In endometriosis group, preoperative CA 125 median serum level was 40.5 KU/L (interquartile range 25% 25 - 75% 112.2), postoperative one was 32.7 KU/L (interquartile range 25% 17.87-75% 65.1).

Preoperative HE4 median serum level was 42.8 pmol/L. (interquartile range 25% 37.9-75% 46.27), postoperative one was 40 pmol/L (interquartile range 25% 36.1-75% 47.2).

In non-endometriosis group, preoperative CA 125 median serum level was 17.6 KU/L (interquartile range 25% 14.27 75% 29.27), postoperative one was 25.4 KU/L (interquartile range 25% 18.57-75% 36.07). Preoperative HE4 median serum level was 44.1 pmol/L. (interquartile range 25% 37.8-75% 49.52), postoperative one was 41.7 pmol/L (interquartile range 25% 38.57-75% 48.72). In the control group, CA 125 median serum level was 14.6 KU/L (interquartile range 25% 11.5 75% 22.2), while HE4 median serum level was 36.6 pmol/L (interquartile range 25% 29.9-75% 40.5).

In patients with endometriosis, benign pelvic mass, and healthy controls, HE4 median serum level was below the 70 pmol/L limit. In the endometriosis group, CA 125 median serum level was significantly higher than in the control group and it decreased one month after surgery. In non-endometriosis group, CA 125 median serum level increased significantly after surgery.

The results were analyzed with Dunn's Method for multiple comparison procedures: comparing the endometriosis and non-endometriosis group, pre- and post-surgery HE4 serum levels did not statistically differ. Comparing these two groups, preoperative CA125 serum levels showed a statistically significant difference, while no difference was found in the postoperative period. The values are shown in Table 2. None of the patients required laparotomic procedure and none of the patients had diagnosis of malignancy at the histopathological specimen analysis.

Table 2. — Median Ca125 HE4 levels in different groups

|                       | Ovarian benign cyst (n=13) | Endometriosis (n=25) | Controls (n=26) | p value  |
|-----------------------|----------------------------|----------------------|-----------------|----------|
| Ca 125 (KU/L)         | 17.6                       | 40.5                 | 14.6            | < 0.001* |
| Pre-operative         |                            |                      |                 |          |
| 1month post-operative | 25.4                       | 32.7                 | 14.6            | < 0.001° |
| HE4 (pmol/L)          | 44.1                       | 42.8                 | 36.6            | < 0.05^  |
| Pre-operative         |                            |                      |                 |          |
| 1month post-operative | 41.7                       | 40.7                 | 36.6            | < 0.05#  |

\* CA 125 endometriosis vs controls  $p < 0.05$ ; endometriosis vs benign cyst  $p < 0.05$ ; benign cyst vs controls  $p > 0.05$ .

° CA 125 endometriosis vs controls  $p < 0.05$ ; endometriosis vs benign cyst  $p > 0.05$ ; benign cyst vs controls  $p > 0.05$ .

^ HE4 benign cyst vs control  $p < 0.05$ ; benign cyst vs endometriosis  $p > 0.05$ ; endometriosis vs controls  $p < 0.05$ .

# HE4 benign cyst vs controls  $p < 0.05$ ; benign cyst vs endometriosis  $p > 0.05$ ; endometriosis vs controls  $p < 0.05$ .

## Discussion

Ovarian cysts in fertile women are a quite common findings: in most cases requiring surgery, ovarian masses are endometriomas (35% of cases) [2]. When an endometrioma was found at ultrasound investigation, an accurate examination of the pelvis by means of multisite ultrasonographic examinations is mandatory to rule out other endometriotic nodules [12-14].

Endometriosis genesis is still matter of concern and several theories were proposed to explain it [15, 16]. Inflammation plays a key role in tissue uptake of endometriosis cells: the involvement of the phosphoinositide-specific phospholipase C (PI-PLC) enzymes, corroborate the hypothesis that phosphoinositide signal (PI) may be involved in the pathogenesis of endometriosis [17].

Tissue architecture of ectopic lesions shows a significant heterogeneity and abnormalities, compared to normal endometrium features [18]. In endometriosis, eutopic endometrium, biochemical and ultrastructural features may also present a significant difference than those of normal endometrium. They are particularly marked in the mild-secretory part of the cycle: these findings may explain the association between endometriosis and infertility [19].

Epidemiologic, histopathologic, and molecular data suggest that endometriosis has a malignant potential [4]. The most frequently used marker for ovarian cancer is CA 125: it is a high weight molecular glycoprotein, also present in the epithelium of fallopian tubes, endometrium, endocervix, peritoneum, pleura, and pericardium [20].

Elevated serum levels of CA 125 are not only associated to endometriosis, but also to many conditions, such as pelvic inflammatory disease, ovarian hyperstimulation, fibroids, pregnancy [15], tuberculosis, cirrhosis, and other carcinomas. It could help to distinguish, with a high false positive and a low sensitivity, early – stage of ovarian cancer from benign gynaecological conditions [7,8]. CA 125 concentration varies on the phases of menstrual cycle [21] and it decreases during treatment with danazol and leuporelin acetate [22].

HE4 is a new tumor marker: its gene expression is amplified in ovarian carcinomas than in normal tissue [23].

A total eradication of the lesion should be performed, minimizing the damage to the ovarian reserve, and avoiding consequent disease relapse due to incomplete treatment hence [24].

Several studies comparing CA 125 and other markers show the necessity of a really sensitive blood test marker for endometriosis. Tokmak *et al.* [6] compared Urocortin and CA 125: they did not find Urocortin to be effective as or more than CA 125 in distinguish endometrioma from other benign cysts. Mohamed *et al.* [25] compared CA 125 to VEGF-A and concluded that VEGF-A is better than CA 125 for the diagnosis and the follow-up of ad-

vanced endometriosis after conservative laparoscopic surgery.

Since HE4 has been identified as the molecular candidate for early stages of ovarian cancer [26], it is less frequently raised in patients with non malignant ovary disease [23]. HE4 may have a relative low specificity: in fact, some pulmonary, endometrial, and breast adenocarcinomas can express it [27].

Even if elevated serum CA 125 concentrations are associated with the presence of moderate to severe endometriosis [28], its utilization in the detection of endometriosis has shown a specificity of 85% and a sensitivity between 20% and 50% [29].

In the present series, comparing HE4 and CA125, the authors did not observe differences of HE4 serum levels in the three study group: before and after surgery its value remained under the cutoff. CA 125 was slightly higher in the endometriosis group before surgery and it decreased one month after surgery. This result was related to the effectiveness of the surgical approach. In the non-endometriosis group, the authors recorded an unexpected increase of CA 125 after surgery: it might be due to the inflammatory reactions which may alter the peritoneal permeability.

In the present study, all the patients subjected blood samples in the early proliferative phase of the cycle in order to minimize CA125 fluctuations: in menstrual period CA125 mean concentrations, could be significantly higher in endometriosis patients than in control group [30]. On the other hand, HE4 serum levels measurements can be performed at any phase of the menstrual cycle [31].

It could help to better discriminate patients with ovarian malignancies from those with endometriomas, as reported by Huhtien *et al.* [10]. As previously asserted in literature [32], the present authors confirmed HE4 and CA 125 combination could differentiate ovarian endometriosis from ovarian malignant masses but in the pre-surgery work up of low risk ovarian cysts, HE4 is not useful.

Given that pre- and post-surgery CA125 serum assay can help to detect the recurrence of endometrioma, while HE4 normal levels are strongly suggestive of a benign condition [33], in the authors' opinion, its dosage is unnecessary in patients with adnexal pathology without suspect of malignancy and would increase healthcare spending.

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