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# Utility of Human Epididymis protein 4, Risk of Malignancy Algorithm to differentiate Ovarian Malignancy from endometriosis in women with adnexal mass of suspected malignancy

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**Abstract**

**Background and Objectives**:
Endometriosis is a benign gynecological disease that displays symptoms and pathophysiological features similar to ovarian carcinoma.CA-125, a well Known biomarker of ovarian malignancy often shows false positive results in Endometriosis resulting many women undergo unnecessary surgery. The aim of our study is to evaluate utility of HE4, ROMA to differentiate endometriosis from ovarian cancer.

**Methodology**: 90 Women having adnexal mass with suspected malignancy on imaging (Ultrasound) anticipated to go for surgery were chosen as patients. Taking Biopsy as gold standard patients were divided into Endometriosis, benign pathology and malignant group.Levels of biomarkers CA-125 and HE4 were analysed, Risk of malignancy algorithm (ROMA) was calculated and compared with histopathology report. Sensitivity, Specificity and Area under Curves were evaluated.

**Results**: Levels of HE4 and ROMA showed a significant increase only in malignant cases ($p \leq 0.001$). But, did not increase significantly in cases with endometriosis and benign pathology ($p \geq 0.05$). Thus, HE4 and ROMA can differentiate endometriosis patients from malignant patients. On the other hand, CA-125 showed a significant increase in malignant as well as endometriosis cases compared to benign pathology ($p \leq 0.001$) but did not showed statistical significant difference between Endometriosis vs. malignant ($p \geq 0.05$).HE4 and ROMA showed specificity of (90.9%) more than CA-125 (54.5%). The Area under Curve of HE4 and ROMA was (0.975 and 0.972) respectively was more than CA-125 (0.753) for distinguishing endometriosis from ovarian malignancy.

**Conclusion**: To Improve diagnosis of women with Endometriosis from Ovarian malignancy, HE4 and ROMA can prove as better markers when CA-125 shows raised values and diagnosis is inconclusive.

Key words: Endometriosis; HE4; ROMA (Risk of Malignancy Algorithm); CA-125; Ovarian Cancer.

## INTRODUCTION

Endometriosis is a benign condition, commonly found in females having incidence rate of approximately 5-10%.[1]Endometrium like cells identical to cells lining inside of uterus grows ectopically leading to an inflammatory response involving activation of macrophages, increased production of cytokines and growth factors in peritoneal fluid. The pathophysiological features, symptoms exhibit similarity with ovarian carcinoma. Patients with endometriosis are at 2.5-4 times increased risk of developing ovarian cancer within 10 years.[2] Endometriosis often presents with a pelvic

mass and raised CA-125 producing a diagnostic dilemma of Ovarian Cancer.CA-125 is commonly used biomarker for endometriosis and is unable to distinguish endometriosis from early stage Ovarian cancer. [3]Transvaginal ultrasound is considered as gold standard for detecting malignancy in adnexal masses. But, in 25% of cases, the diagnosis is inconclusive causing significant emotional stress to the patient.[4]The accurate diagnosis of endometriosis is done by laparoscopy allowing visualization of the lesion and confirmed using histopathology.[5]Women of reproductive age with desire of pregnancy suffers from anxiety of ovarian health damage. So, there is critical need of a diagnostic marker that can successfully differentiate ovarian malignancy from ovarian endometriosis.[6].

Human Epididymis protein 4 is well known marker these days for ovarian malignancy detection. It is a glycoprotein ,protease inhibitor that is involved in maturation of sperm .[7] This prototype of HE4 gene is encoded by WFDC2 gene and has WAP type two four disulfide core domain (WFDC).[8] The gene span 8-12 kb DNA and has five axons. The gene is situated on chromosome 20q12-q13.HE4 is effective in distinguishing endometriosis from early stage of the disease. Its levels are not raised in any stage of endometriosis. Whereas, CA-125 shows sharp elevation in endometriosis women. CA-125 does not display satisfactory specificity especially in premenopausal women affecting authenticity of results. So, clinically raised serum levels of CA-125 and normal HE4 can be helpful in differentiating endometriosis from ovarian cancer.[9]

ROMA, Another proposed model, called the Risk of Ovarian Malignancy algorithm (ROMA) is known to estimate the risk of epithelial ovarian cancer in females possessing pelvic masses. It includes HE4 and CA-125 values into an algorithm depending upon menopausal status. ROMA may benefit in sorting women to an expert gyaecologist.[10]
The Aim of our study is to find utility of HE4 and ROMA to differentiate endometriosis from Ovarian cancer.

**Subjects and Methods**

Study Design: This observational study was done at Department of Biochemistry, of a tertiary care hospital Guru Gobind Singh Medical College and Hospital, Faridkot.

Study Population: The samples were collected over a period of 2 years from 90 patients 18 years or above diagnosed with an adnexal mass of suspected malignancy by imaging, admitted for surgical removal in the Department of Gynaecology and obstetrics of same institution. Patients with previous history of cancer, chemotherapy, pregnancy, concomitant diseases like chronic liver disease, chronic heart failure, and any renal disease were excluded. The Approval from the Institutional Ethical Committee vide letter no. GGS/IEC/18/83 was taken before carrying out the study. The patients were enrolled after taking prior informed consent.

Sample Collection: 5ml venous blood was taken out preoperatively. Blood was allowed to clot and serum was extracted after centrifuging it. Analysis of biomarker CA-125 was done by chemiluminescent immunoassay on a fully automated Access II analyzer (Beckman Coulter, Inc., United States).The cut-off for CA-125 was taken as 35U/ml.

HE4 was analysed by the ELISA kit method (Calbiotech, Inc., EL Cajon, California, United States).The cut-off of HE4 was taken as 70pmol/L and140pmol/L for premenopausal and postmenopausal women respectively.

ROMA is calculated as follows:
Before menopause: $PI = -12.0 + 2.38 \times LN [HE4] + 0.0626 \times LN [CA125]$
After menopause: $PI = -8.09 + 1.04 \times LN [HE4] + 0.732 \times LN [CA125]$
ROMA value (%) = ePI/[1 + ePI] x 100%
Here, PI -predictive index, LN - natural logarithm, e - base of natural logarithm
ROMA cut-offs for women at high risk was taken as >13.1 for premenopausal and >27.7 for postmenopausal women as recommended by Moore et.al [10]

Data Analysis: Data analysis was done using SPSS 21. Based on histopathology, Patients were divided into categories: Endometriosis, benign pathology and malignant groups. Comparison of endometriosis patient was done against benign pathology and malignant group by Kruskal-wallis and Tukey's HSD test. For all statistical analysis, p value of $<0.05$ is taken as statistically significant. The Sensitivity, Specificity was calculated and ROC was (Receiver operating Characteristic Curves) constructed and AUC (Area under the Curve) at 95% CI was calculated for HE4, ROMA and CA-125.

**Results**

The study population included 90 women with adnexal mass scheduled to undergo surgery for removal of mass. Based on results of biopsy three groups were formed: Endometriosis, Benign pathology and malignant group.It was observed that in Benign pathology group (n=43), 30 women were premenopausal and 13 were postmenopausal and in Endometriosis group (n=11), 7 women were premenopausal, 4 were postmenopausal. Out of 36 women with malignant disease, 12 women were premenopausal and 24 were Postmenopausal. So, most of benign masses were found in premenopausal group and malignant masses were found in postmenopausal group. Table 1 shows age related distribution of patients in endometriosis, benign pathology and malignant group. The mean age of total women, Endometriosis,

Benign pathology and malignant group were 46.92±12.71, 47.27±13.68, 43.81±11.70 and 50.53±12.95 respectively. No significant difference in age between the groups was seen (p>0.05) Table1

Table: 1 Shows age related distribution of patients in Endometriosis, Benign pathology and Malignant groups.

| Sr.no. | Groups | Number of patients | Age Mean ± SD Range | S.E | *p*-value |
|---|---|---|---|---|---|
| 1 | Endometriosis | 11 | 47.27± 13.68 (27-70) | 4.12 | >0.05 |
| 2 | Benign pathology | 43 | 43.81± 11.70 (18-70) | 1.78 | |
| 3 | Malignant | 36 | 50.53±12.95 (20-72) | 2.16 | |

Table: 2 Shows Mean and Median levels of all parameters in three groups

| Sr.no. | Parameters | Groups | Median | Mean ± S.D | S.E | *p*-value |
|---|---|---|---|---|---|---|
| 1 | HE4 | Endometriosis | 58 | 64.85±10.35 | 10.35 | 0.001 |
| | | Benign pathology | 56 | 63.35±4.98 | 4.98 | |
| | | Malignant | 199 | 262.04±30.45 | 30.45 | |
| 2 | CA-125 | Endometriosis | 45.9 | 226.11±16.10 | 16.10 | 0.001 |
| | | Benign pathology | 15.52 | 49.12±107.41 | 107.41 | |
| | | Malignant | 259.8 | 735.69±24.00 | 24.00 | |
| 3 | ROMA | Endometriosis | 13.3 | 17.70±43.52 | 43.52 | 0.001 |
| | | Benign pathology | 9.8 | 12.97±873.88 | 873.88 | |
| | | Malignant | 82.25 | 76.38±397.38 | 397.38 | |
| Abbreviations:HE4:Human Epididymis Protein 4, CA-125:Carbohydrate antigen-125, ROMA: Risk Of Malignancy Algorithm, S.E-Standard Error | | | | | | |

The mean and median levels of all parameters in the three groups showed statistical significant difference. (Table 2) When multiple comparisons were made between the groups (Table 3) HE4 did not showed statistical significant difference between Benign pathology group vs. Endometriosis group (p>0.05) but a significant difference was seen between HE4 levels of Benign pathology group vs. Malignant and Endometriosis vs. Malignant group (p<0.001).

CA-125 showed statistically significant difference only between Benign pathology vs. Malignant (p<0.001) but did not showed statistical significant difference between Endometriosis vs. Malignant and Benign pathology group p>0.05.

ROMA showed statistically significant difference between Benign pathology vs. Malignant and Endometriosis vs. Malignant group (p<0.001) but did not showed statistical significant difference between Endometriosis vs. Benign pathology group p>0.05.

Table 4 showed the Sensitivity, Specificity, and Area under curve of CA-125, HE4 and ROMA. We found that HE4 and ROMA had better specificity than CA-125. Though, Sensitivity of all parameters was comparable. HE4 with AUC (area under curve) 0.975 and ROMA with AUC 0.972 were better in performance than CA-125 with AUC 0.753 in distinguishing endometriosis from ovarian cancer. (Fig 1)

It signifies that HE4 and ROMA can differentiate but CA-125 cannot differentiate endometriosis from Ovarian Malignancy. Hence, of limited use in relieving dilemma of women with endometriosis from malignancy. Our data shows that HE4 and ROMA are better markers in distinguishing Endometriosis from Ovarian Malignancy.

Table 3: Shows comparisons between groups

| Sr.No. | Parameters | Group(1) | Group(2) | Mean difference (Gr.1-Gr.2) | S.E | p-value |
|---|---|---|---|---|---|---|
| 1. | HE4 | endometriosis | Benign pathology | 1.49 | 40.09 | >0.05 |
| | | | malignant | -197.199* | 40.88 | <0.001 |
| | | Benign pathology | endometriosis | -1.49 | 40.09 | >0.05 |
| | | | malignant | -198.690* | 26.8 | <0.001 |
| 2. | CA-125 | endometriosis | Benign | 176.99 | 263.3 | >0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | pathology | | | |
| | | | malignant | -509.58 | 268.46 | >0.05 |
| | | Benign pathology | endometriosis | -176.99 | 263.3 | >0.05 |
| | | | malignant | -686.572* | 176.04 | <0.001 |
| 3 | | endometriosis | Benign pathology | 4.73 | 5.86 | >0.05 |
| | | | malignant | -58.681* | 5.97 | <0.001 |
| | | Benign pathology | endometriosis | -4.73 | 5.86 | >0.05 |
| | ROMA | | malignant | -63.406* | 3.92 | <0.001 |
| *Abbreviations*:HE4:Human Epididymis Protein 4, CA-125: Cancer antigen-125, ROMA: Risk Of Malignancy Algorithm, S.E-Standard Error | | | | | | |

Table 4: Shows Sensitivity, Specificity, Area under curve of Cancer Antigen-125, Human Epididymis Protein 4 and Risk of Malignancy Algorithm

| Sr. No. | Parameter | Sensitivity | Specificity | Area Under Curve | S.E | *P*-Value |
|---|---|---|---|---|---|---|
| 1. | HE4 | 97.2 | 90.9 | 0.975 | 0.024 | <0.001 |
| 2. | ROMA | 100 | 90.9 | 0.972 | 0.028 | <0.001 |
| 3. | CA-125 | 97.2 | 54.5 | 0.753 | 0.093 | <≤0.05 |
| *Abbreviations*:HE4:Human Epididymis Protein 4, CA-125:Cancer antigen-125, ROMA: Risk Of Malignancy Algorithm, S.E-Standard Error | | | | | | |

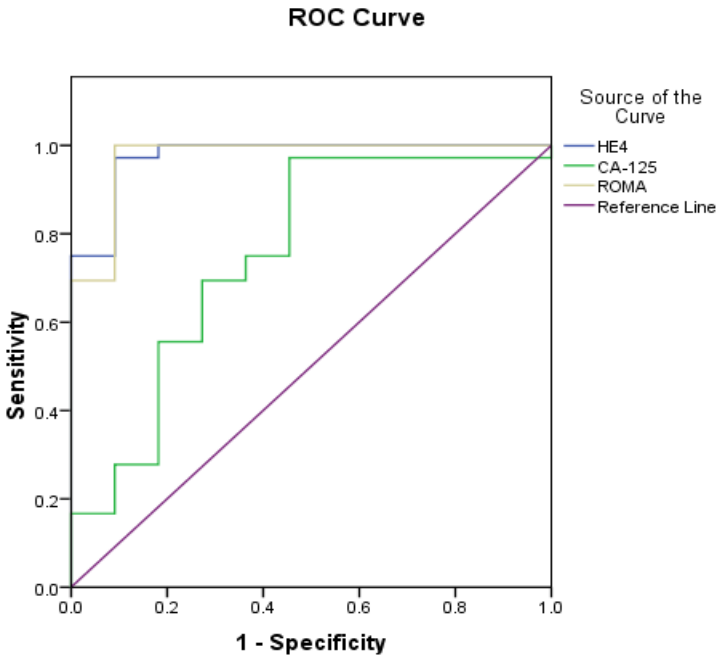


Fig 1: Area Under Receiver Operating characteristic curve (ROC) of CA-125, HE4, ROMA Endometriosis vs. Malignant. *Abbreviations*: HE4: Human Epididymis Protein 4, CA-125: Cancer antigen-125, ROMA: Risk Of Malignancy Algorithm

**Discussion**

Ovarian cancer is fatal among various gynecological malignancies because of its initial no symptom, silent nature of development so, usually detected at an advanced stage where prognosis is poor. Endometriosis is a benign disease displaying features similar to ovarian malignancy like increased angiogenesis, abnormal patterns of apoptosis, uncontrolled cellular proliferation and invasion of distant organs etc. and thus deteriorating emotional health of many women due to associated anxiety.[11,12]CA-125 is widely known biomarker for diagnosis of Ovarian malignancy in patients having an adnexal mass. But, in various benign conditions CA-125 showed false elevation as its levels are non-specifically raised in conditions like endometriosis, pelvic inflammatory disease, fibroids, infections etc.[13] It is quite challenging to differentially diagnose endometriosis from ovarian cancer as often ultrasound features are difficult to interpret and raised CA-125 is seen in both conditions. For an optimal surgical approach, preoperative risk assessment is needed. This study examines the performance of CA-125, HE4, and ROMA to differentially diagnose endometriosis from ovarian malignancy. A considerable number of premenopausal women undergo unnecessary surgery as most of the women with raised CA-125 are referred to oncology units .[14] Our study showed HE4 did not rise significantly in

endometriosis group whereas CA-125 rise significantly. It was shown by many researches that increase of HE4 in benign gynaecologic conditions is rare and suggested a complementary role to CA125.[15, 16] In women with endometriosis, Moore et al. observed that HE4 showed increase in only 3% of women whereas CA-125 showed increase in 67% of patients.[16] Antasi et.al suggested that determination of HE4 is best approach to confirm benign nature of endometrioma where CA-125 showed higher levels [17].

Our study showed increased specificity of HE4 and ROMA compared to CA-125. It is supported by some recent studies.[18] In our study The AUC of HE4 in patients with endometriosis compared to benign was 0.975 better than CA-125 AUC(0.753).It is supported by study of Braicu EI et.al that showed performance of HE4 is better than CA-125 alone with an AUC of 0.912 (95% CI 0.872–0.953) and 0.812 (95% CI 0.753– 0.871).[15] In our study, the AUC of ROMA did not showed significant difference from HE4(0.972 and0.975).These findings are similar to study of Chen et.al.[19]

**Conclusion**

In our study HE4 performed better by displaying best specificity and AUC. It can help to distinguish between endometriosis and ovarian cancer patients when results of ultrasound and CA-125 are inconclusive. Though our study involves a limitation like population in endometriosis group was small but results are worth noting.

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