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Research Article

Serum HE4 and CA-125 as Combined Biomarkers for Early Diagnosis of Ovarian Carcinoma

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Abstract: Early detection of epithelial ovarian carcinoma remains a major clinical challenge due to its asymptomatic progression and lack of reliable single diagnostic biomarkers. This prospective case–control study evaluated the diagnostic performance of serum human epididymis protein 4 (HE4) and cancer antigen 125 (CA-125) individually and in combination for early-stage ovarian carcinoma. A total of 240 women were enrolled between January 2022 and May 2024, comprising 120 histopathologically confirmed ovarian carcinoma cases (stages I–II: n = 68; stages III–IV: n = 52) and 120 age-matched controls with benign ovarian lesions. Serum HE4 and CA-125 were quantified using electrochemiluminescence immunoassay, and diagnostic performance was assessed via receiver-operating characteristic (ROC) analysis. Mean HE4 and CA-125 levels were significantly higher in malignant compared to benign groups ($p < 0.001$). The area under the ROC curve (AUC) was 0.91 for HE4, 0.88 for CA-125, and 0.96 for the combined model. The combined markers achieved sensitivity of 92.6% and specificity of 90.3% for early-stage disease, significantly outperforming either biomarker alone ($p < 0.001$). These findings demonstrate that concurrent assessment of HE4 and CA-125 substantially enhances early diagnostic accuracy and may improve clinical triage and surveillance protocols.

Keywords: HE4, CA-125, ovarian carcinoma, early detection.

Introduction: Ovarian carcinoma remains one of the most lethal malignancies affecting women worldwide, primarily due to its silent onset and late diagnosis. Despite advances in surgical and

chemotherapeutic management, the five-year survival rate for advanced-stage disease remains below 30%, whereas early-stage detection dramatically improves outcomes, exceeding 90% survival when confined to the ovary. Unfortunately, less than one-third of cases are diagnosed at an early stage, underscoring the urgent need for sensitive and specific biomarkers capable of identifying malignancy before metastasis or peritoneal spread.1-3

Historically, cancer antigen 125 (CA-125) has been the most widely used biomarker for ovarian cancer detection and monitoring. CA-125 is a high-molecular-weight glycoprotein expressed on coelomic epithelial derivatives, including the Müllerian epithelium. While elevated CA-125 levels are observed in approximately 80% of advanced epithelial ovarian cancers, its sensitivity for stage I disease is considerably lower (around 50%), and false elevations occur in benign conditions such as endometriosis, pelvic inflammatory disease, and menstruation. This lack of specificity limits its utility as a standalone screening tool for early diagnosis.4-6

Recent advances have identified human epididymis protein 4 (HE4) as a promising complementary biomarker. HE4, a member of the whey acidic protein four-disulfide core family, is overexpressed in serous and endometrioid epithelial ovarian carcinomas but rarely elevated in benign gynecologic disorders. Unlike CA-125, HE4 expression is less influenced by menstrual cycle or benign inflammation, thereby enhancing specificity. Several multicentric analyses since 2022 have demonstrated that HE4 exhibits superior specificity (90–95%) and better discrimination between malignant and benign adnexal masses than CA-125 alone.7-10

The integration of HE4 and CA-125 into risk algorithms such as the Risk of Ovarian Malignancy Algorithm (ROMA) has improved diagnostic performance in Western populations. However, ethnicity, environmental factors, and genetic predispositions influence biomarker expression; thus, regional validation in South Asian cohorts remains necessary. There is a paucity of recent large-scale prospective data evaluating the combined diagnostic potential of HE4 and CA-125 in Pakistani women, where ovarian malignancy often presents at an advanced stage due to limited access to specialized screening.11-13

In the last few years, studies employing multiparametric biomarker panels have shown that combined serum markers outperform imaging or single assays in differentiating malignant from

benign adnexal lesions. Integrating HE4 and CA-125 has been particularly effective in detecting early-stage disease and improving predictive accuracy in postmenopausal women. A growing body of literature published between 2022 and 2024 supports the combined use of HE4 and CA-125 to enhance sensitivity without compromising specificity. However, clinical translation requires population-specific cut-off optimization and evaluation of diagnostic accuracy within distinct demographic and biological settings.

The present study was therefore designed to evaluate serum HE4 and CA-125 levels among patients with histologically confirmed ovarian carcinoma and benign ovarian masses. The objective was to assess the individual and combined diagnostic efficacy of these biomarkers, determine optimal cut-off values for early-stage disease detection, and validate their synergistic performance through ROC curve analysis. It was hypothesized that concurrent measurement of HE4 and CA-125 would significantly improve early diagnostic precision compared with either marker alone, providing a cost-effective strategy adaptable to regional screening programs.

Methodology: This prospective, case–control observational study was conducted at THQ Hospital, Shujabad, Multan the Department of Obstetrics and Gynecology in collaboration with CMH Lahore Medical College. Ethical approval was obtained from the institutional review committee, and verbal and written informed consent was secured from all participants.

A total of 240 women aged 25–70 years were enrolled and categorized into two groups: Group I (cases)—120 patients with histopathologically confirmed epithelial ovarian carcinoma, and Group II (controls)—120 age-matched women with benign ovarian cysts confirmed by imaging and histology. Exclusion criteria included pregnancy, prior chemotherapy or radiotherapy, renal failure, and concurrent malignancies.

Sample size was calculated using Epi Info (version 7) software, based on an expected sensitivity of 85% for combined HE4 + CA-125, precision of 5%, confidence level of 95%, and expected disease prevalence of 2%, yielding a minimum of 216 participants; the final sample was increased to 240 to account for potential dropouts.

Venous blood samples (5 mL) were collected preoperatively under aseptic conditions, centrifuged, and serum stored at −80°C until analysis. Serum HE4 and CA-125 concentrations were measured

by electrochemiluminescence immunoassay (ECLIA) using standardized calibrations. Reference ranges were set at ≤70 pmol/L for HE4 and ≤35 U/mL for CA-125.

Statistical analysis was performed using SPSS version 27. Data normality was assessed by the Shapiro–Wilk test. Quantitative variables were expressed as mean ± standard deviation and compared using independent-sample t-tests. Diagnostic sensitivity, specificity, and predictive values were derived, and ROC curves were generated to calculate AUCs. Logistic regression assessed combined marker performance, with $p < 0.05$ considered statistically significant.

Results

Table 1. Demographic and clinical characteristics of study participants (n = 240)

Parameter	Malignant (n=120)	Benign (n=120)	p-value
Mean age (years)	53.4 ± 10.2	48.1 ± 9.5	0.003
Postmenopausal (%)	62.5	41.7	0.001
Mean tumor size (cm)	7.8 ± 2.4	4.1 ± 1.6	<0.001

Table 1 shows that malignant cases were older, predominantly postmenopausal, and had significantly larger tumor sizes than benign lesions.

Table 2. Mean serum biomarker levels in study groups

Biomarker	Malignant (mean ± SD)	Benign (mean ± SD)	p-value
HE4 (pmol/L)	186.2 ± 75.8	61.7 ± 28.9	<0.001
CA-125 (U/mL)	243.5 ± 108.6	48.3 ± 24.7	<0.001

Both serum HE4 and CA-125 levels were markedly higher in ovarian carcinoma patients, with differences statistically significant ($p < 0.001$).

Table 3. Diagnostic performance of biomarkers for early-stage (I–II) ovarian carcinoma

Biomarker	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HE4	0.91	85.3	88.1	86.2	87.4

Biomarker	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CA-125	0.88	80.1	85.7	82.4	84.2
Combined (HE4 + CA-125)	0.96	92.6	90.3	91.1	92.0

Table 3 indicates that combined HE4 and CA-125 achieved the highest diagnostic accuracy (AUC 0.96), outperforming either biomarker alone for early-stage disease.

Discussion: This study demonstrated that combined measurement of serum HE4 and CA-125 significantly enhances the early diagnostic accuracy for epithelial ovarian carcinoma compared with single-marker assessment. The sensitivity and specificity achieved by the combined model (92.6% and 90.3%, respectively) exceed those reported for either HE4 or CA-125 alone, aligning with recent multi-institutional data emphasizing the complementary nature of these biomarkers.13-14

HE4 exhibited superior specificity compared with CA-125, confirming its robustness in distinguishing malignant from benign adnexal lesions. CA-125, though sensitive, is often confounded by benign gynecologic conditions and inflammatory states. The integration of HE4 mitigates this limitation by providing specificity derived from tumor-specific overexpression, particularly in serous and endometrioid histotypes.15-17

The elevated mean levels of both markers among postmenopausal women reinforce age- and hormonal-related expression differences, consistent with current literature. Notably, combining HE4 with CA-125 yielded an AUC of 0.96, suggesting near-optimal discrimination. These results validate the biological premise that independent molecular pathways underpin HE4 and CA-125 secretion, providing complementary diagnostic information.18-20

Several investigations between 2022 and 2024 have supported the superiority of dual-marker algorithms over traditional CA-125 testing, especially for early-stage disease. Studies employing ROMA scoring algorithms have demonstrated comparable AUC values ranging from 0.94 to 0.97, further corroborating the high diagnostic yield of combined biomarkers.

In resource-limited settings, a dual-biomarker strategy offers a pragmatic approach to early screening, potentially reducing unnecessary laparotomies and facilitating timely referral for oncologic management. Implementing serum HE4 and CA-125 testing as a combined triage tool could improve preoperative stratification and guide the need for advanced imaging or surgical exploration.

The prospective design, histopathological confirmation, and population-specific sample strengthen the validity of these findings. However, single-centre recruitment and exclusion of non-epithelial malignancies limit generalizability. Longitudinal follow-up to correlate biomarker kinetics with recurrence and treatment response would further enhance clinical applicability.

Overall, the data underscore that the combined assessment of HE4 and CA-125 represents a superior diagnostic model, capable of distinguishing malignant from benign ovarian disease with high accuracy, particularly in early stages when intervention has the greatest prognostic impact.

Conclusion: Concurrent measurement of serum HE4 and CA-125 substantially improves sensitivity and specificity for early ovarian carcinoma detection compared with single-marker assays. This combined biomarker strategy offers a feasible, cost-effective tool for early diagnosis, bridging existing gaps in current screening protocols and guiding future development of multimodal diagnostic algorithms.

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