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Symptom-Based Score, CA125, and HE4 for Prediction of Ovarian Cancer in Iranian Women with Adnexal Mass

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ABSTRACT

Background & Objective: Ovarian cancer is a leading cause of gynecologic cancer mortality. Differentiating benign from malignant adnexal masses remains a key clinical challenge. This study was conducted with aim to evaluate a symptom-based score, CA125, and HE4, both individually and combined, for diagnosing ovarian cancer in Iranian women.

Materials & Methods: This prospective analytical study was conducted in 2024 at Imam Hossein Hospital in Tehran. A total of 84 women with adnexal masses confirmed by MRI and scheduled for surgery were enrolled. Preoperative symptom scores and serum CA125 and HE4 were measured. Pathological diagnosis was the gold standard. Predictive power was assessed using logistic regression and diagnostic performance metrics (sensitivity, specificity, AUC). Data analysis was performed using Stata (version 17.) P<0.05 was considered statistically significant.

Results: Of the 84 participants, 31(36.9%) had malignant tumors, 8(9.5%) borderline, and 45(53.6%) benign tumors. The malignant group was significantly older and had significantly higher levels of CA125, HE4, and symptom scores (P<0.001 for all biomarkers and score). In the combined multivariate model, only the symptom score remained an independent significant predictor (OR: 1.62, 95% CI: 1.24-2.13). The combined model achieved the highest discriminative ability (AUC: 0.971). The symptom score alone demonstrated the most balanced diagnostic profile with 83.87% sensitivity, 92.45% specificity, 90.74% NPV, and 89.29% accuracy.

Conclusion: The symptom-based score demonstrated superior individual predictive performance for ovarian cancer compared to CA125 or HE4 alone. Although the three-marker combination yielded the highest discriminative power, the symptom score offered an optimal balance of high sensitivity and accuracy for practical clinical use.

Keywords: Ovarian cancer, Symptom score, CA125, HE4, Adnexal mass, Diagnosis

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1. Introduction

Ovarian cancer is the seventh most common cancer globally, with 294,000 new cases annually (1). It is the most common gynecologic cancer after cervical and uterine cancers (2). Ovarian cancer has the highest mortality rate among gynecologic malignancies, is associated with poor prognosis and low survival rates (3,4). It is estimated that by 2040, the incidence of ovarian cancer will increase significantly (5). Statistics show that the mortality rate from ovarian cancer increased by 84.2% between 1990 and 2017 (6). In Iran, this cancer is the eighth most common cancer with an age-standardized incidence rate of 3.9 per 100,000 women (7). According to a study that examined 7,977 cases of ovarian cancer from 2009 to 2014, the 5-year survival rate in Iran was reported as 55% and the 10-year survival rate as 45% (8).

Diagnosing ovarian cancer is challenging due to the low prevalence of the disease (9,10). Although women at high risk can be identified and benefit from intensive screening, only 10% of ovarian cancer cases occur in this group (11). The combination of CA125 and Transvaginal Ultrasound (TVS) is recommended for screening high-risk women, but TVS as a primary screening tool may have high sensitivity but also produces a high rate of false-positive results (12). Furthermore, CA125 is elevated in only 50% of patients with early-stage ovarian cancer, and efforts are

ongoing to improve the performance of this marker and identify new biomarkers **(13)**.

Another promising marker is Human Epididymis Protein 4 (HE4), which is specifically expressed in ovarian carcinomas **(14)**. HE4 has been proposed as a potential marker for ovarian cancer and, when combined with CA125, increases the accuracy of predicting malignancy in ovarian tumors **(15)**. Previously, it was thought that symptoms of ovarian cancer only appeared in advanced stages, but it is now known that women with early-stage disease may also report non-specific symptoms. Consequently, a Symptom Index (SI) has been developed that can help identify women with ovarian cancer. Using HE4, CA125, and SI as components of a multi-stage screening program can provide acceptable sensitivity and specificity **(16)**.

The present study was conducted with aim to evaluate the predictive value of combining a symptom-based score, CA125, and HE4 in diagnosing ovarian cancer in Iranian women with adnexal masses. Given the genetic and environmental differences among various populations, investigating the efficacy of these indices in the Iranian female population is of particular importance. The results of this study could contribute to providing a more accurate and efficient diagnostic model for the early detection of ovarian cancer in Iranian women and, ultimately, lead to improved clinical outcomes and enhanced quality of life for these patients.

2. Materials and Methods

This prospective analytical study was conducted in 2024 at Imam Hossein Hospital in Tehran affiliated to Shahid Beheshti University of Medical Sciences. The study population consisted of female patients with confirmed adnexal masses (O-RADS 4) on MRI and ultrasound who were candidates for surgery. The sample size was calculated based on an estimated malignancy prevalence of 30% in patients with O-RADS 4 masses, with a 10% margin of error and 95% confidence level, resulting in a required sample size of 84 participants. Convenience sampling was employed to enroll eligible patients until the target sample size was reached.

Inclusion criteria were: Women aged 18 years or older, presence of adnexal mass confirmed by ultrasound, or MRI with O-RADS 4 score, scheduled for surgical intervention for adnexal mass investigation, and willingness to provide blood samples for biomarker testing. Exclusion criteria included: history of pelvic surgery within the past 6 weeks, current pregnancy or lactatio, previous history of any cancer (except non-melanoma skin cancer), chemotherapy or radiotherapy treatment within the past 6 months, active pelvic inflammatory disease, severe hepatic or renal disorders, active autoimmune diseases, use of medications affecting CA125 or HE4 levels, known metastasis from other cancers to the ovary, and severe psychiatric disorders impairing study cooperation.

After obtaining informed consent, demographic and clinical data were collected using a researcher-made checklist including age, marital status, education level, family history of ovarian and related cancers, menopausal status, history of oral contraceptive use, BMI, pregnancy and childbirth history, detailed clinical symptoms, imaging results, levels of other serum biomarkers, assisted reproductive technology history, and history of underlying diseases.

A symptom-based scoring system was applied to patients preoperatively. The system included seven symptoms: abdominal bloating (2 points), urinary frequency (2 points), rectal bleeding (2 points), postmenopausal bleeding (2 points), loss of appetite (3 points), abdominal pain (3 points), and abdominal distension (5 points). The total score was calculated by summing all points, with an additional point for women aged 50 years or older **(17)**. This scoring was performed by a researcher blinded to imaging findings and tumor marker levels.

Blood samples were collected either at the clinic before surgery or on the day of surgery in the operating room before surgical incision. Samples were maintained at room temperature for at least 30 minutes to clot, then centrifuged at 1200×g for 10 minutes. The separated serum was stored at -80°C. CA125 and HE4 levels were measured using sandwich ELISA method with specific monoclonal antibodies. Normal levels were considered as <35 U/mL for CA125, <70 pmol/L for HE4 in premenopausal women, and <140 pmol/L in postmenopausal women.

Following surgery, the mass samples were sent for pathological examination. Based on pathological results, the patients were divided into three groups: malignant mass (confirmed ovarian cancer), borderline, and benign mass groups. Data comparison and analysis were performed based on pathological outcomes.

The predictive power of the three methods (symptom index, CA125, and HE4) for diagnosing ovarian cancer was evaluated by calculating sensitivity and specificity for each method separately. Logistic regression models were used to assess independent and concurrent predictive power. Independent variables included symptom index, CA125, and HE4, with the dependent variable being the probability of ovarian cancer. ROC curves were plotted for each method, and AUC was calculated. Data analysis was performed using Stata (version 17). $P<0.05$ was considered statistically significant.

3. Results

Of the total 84 patients with adnexal mass, 45(53.6%) had benign tumors, 8(9.5%) borderline

tumors, and 31(36.9%) malignant ovarian tumors. The mean age of patients with malignant tumors (52.58 years) was significantly higher than that of patients with benign (45.64 years) and borderline tumors (40.75 years) (P=0.024). The levels of CA125 and HE4 biomarkers in the malignant group, with means of 456.35 U/mL and 237.68 U/mL, respectively, were significantly higher than those in the benign and borderline groups (P < 0.001 for both). Furthermore, the symptom score in the malignant group (12.23) was significantly higher than in the borderline (7.50) and benign (5.69) groups (P<0.001). No significant differences were observed in body mass index and age at first pregnancy among the three groups (Table 1).

Analysis of clinical and reproductive characteristics revealed a significant association between a positive family history of cancer and tumor pathology (P=0.001), with the malignant group having a substantially higher proportion of affected individuals (35.5%) compared to the benign group (4.4%). Other variables, including marriage status, menopausal status, OCP use, pregnancy history, ART use, or endometriosis, didn't show statistically significant differences across the tumor groups. Notably, the majority of patients in all groups were married and nulliparity was not concentrated in any specific group. The use of assisted reproductive technology was low across all groups, and endometriosis was present in a minority of cases without significant variation by pathology (Table 2).

The diagnostic performance of various prediction models is summarized in Table 3. All univariate models demonstrated significant predictive value for malignant pathology. The symptom score (Model 3) showed the strongest individual association, with an odds ratio of 1.93 (95% CI: 1.48-2.53, P<0.001) and an AUC of 0.910. In the multivariate model combining all three predictors (Model 4), only the symptom score remained statistically significant (OR: 1.62, 95% CI: 1.24-2.13, P<0.001), while CA125 and HE4 lost their independent significance. The combined model achieved the highest discriminative ability with an AUC of 0.971, indicating excellent diagnostic performance for distinguishing malignant from benign adnexal masses.

Table 4 presents the diagnostic performance metrics of the four prediction models at the standard probability cutoff of 0.5. The symptom score alone (Model 3) demonstrated the most balanced performance profile, achieving the highest sensitivity (83.87%), negative predictive value (90.74%), and overall accuracy (89.29%). While the combined model (Model 4) attained the highest discriminative ability (AUC: 0.971), it showed slightly lower sensitivity (77.42%) compared to the symptom score model. Both biomarker-only models (CA125 and HE4) exhibited high specificity (>92%) but substantially lower sensitivity (<62%), resulting in lower overall accuracy (80.95% for both). These findings suggest that the symptom score provides an optimal balance between ruling out and ruling in malignant pathology, while the addition of serum biomarkers primarily enhances the model's overall discriminative power as reflected in the AUC. ROC curves comparison of predictive models for ovarian malignancy is shown in Figure 1.

Table 1. Baseline characteristics and biomarker levels of the participants by tumor pathology

Variable	Benign (Mean±SD)	Borderline (Mean±SD)	Malignant (Mean±SD)	P-value
Age (year)	45.64±13.78	40.75±8.86	52.58±12.49	0.024
BMI (kg/m^2)	28.00±4.25	27.75±3.33	25.71±4.47	0.070
Age at 1st pregnancy (year)	23.89±4.43	26.14±3.72	24.32±5.81	0.552
CA125 U/mL	30.26±40.77	43.50±47.71	456.35±663.82	<0.001
HE4 U/mL	48.60±36.46	48.38±32.94	237.68±235.60	<0.001
Symptom score	5.69±2.31	7.5±4.63	12.23±3.38	<0.001

Table 2. Distribution of clinical and reproductive characteristics by tumor pathology

Variable		Benign N(%)	Borderline N(%)	Malignant N(%)	P-value
Marriage status	Yes	39(86.7)	8(100.0)	29(93.5)	0.379
	No	6(13.3)	0(0.0)	2(6.5)	
Family history of cancer	Yes	2(4.4)	0(0.0)	11(35.5)	0.001
	No	43(95.6)	8(100.0)	20(64.5)	
Menopause	Yes	17(37.8)	1(12.5)	15(48.4)	0.172
	No	28(62.2)	7(87.5)	16(51.6)	
OCP Use	Yes	12(26.7)	4(50.0)	4(12.9)	0.072
	No	33(73.3)	4(50.0)	27(87.1)	
Pregnancy history	0	10(22.2)	1(12.5)	3(9.7)	0.807
	1	2(4.4)	1(12.5)	3(9.7)	
	2	12(26.7)	4(50.0)	7(22.6)	
	3	9(20.0)	0(0.0)	11(35.5)	
	≥4	12(26.7)	2(25.0)	7(22.6)	
ART use	Yes	3(6.7)	1(12.5)	0(0.0)	0.227
	No	42(93.3)	7(87.5)	31(100.0)	
Endometriosis	Yes	4(8.9)	1(12.5)	5(16.1)	0.631
	No	41(91.1)	7(87.5)	26(83.9)	

Table 3. Performance of logistic regression models for predicting malignant ovarian pathology

Model	Variables	Odds Ratio (95% CI)	P-value	AUC-ROC
Model 1	CA125	1.016(1.006-1.026)	0.001	0.865
Model 2	HE4	1.023(1.011-1.035)	<0.001	0.849
Model 3	Symptom score	1.934(1.481-2.525)	<0.001	0.91
Model 4	Combined (CA125+HE4+Symptom score)	CA125: 1.007 (0.997-1.018) HE4: 1.012 (0.997-1.028) Score: 1.624 (1.239-2.13)	CA125: 0.177 HE4: 0.129 Score: <0.001	0.971

Table 4. **Diagnostic performance of predictive models for malignant ovarian pathology**

Model	Variables	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
Model 1	CA125	61.29	92.45	82.61	80.33	80.95	0.865
Model 2	HE4	58.06	94.34	85.71	79.37	80.95	0.849
Model 3	Score	83.87	92.45	86.67	90.74	89.29	0.940
Model 4	Combined (CA125+HE4+Symptom score)	77.42	92.45	85.71	87.50	86.90	0.971

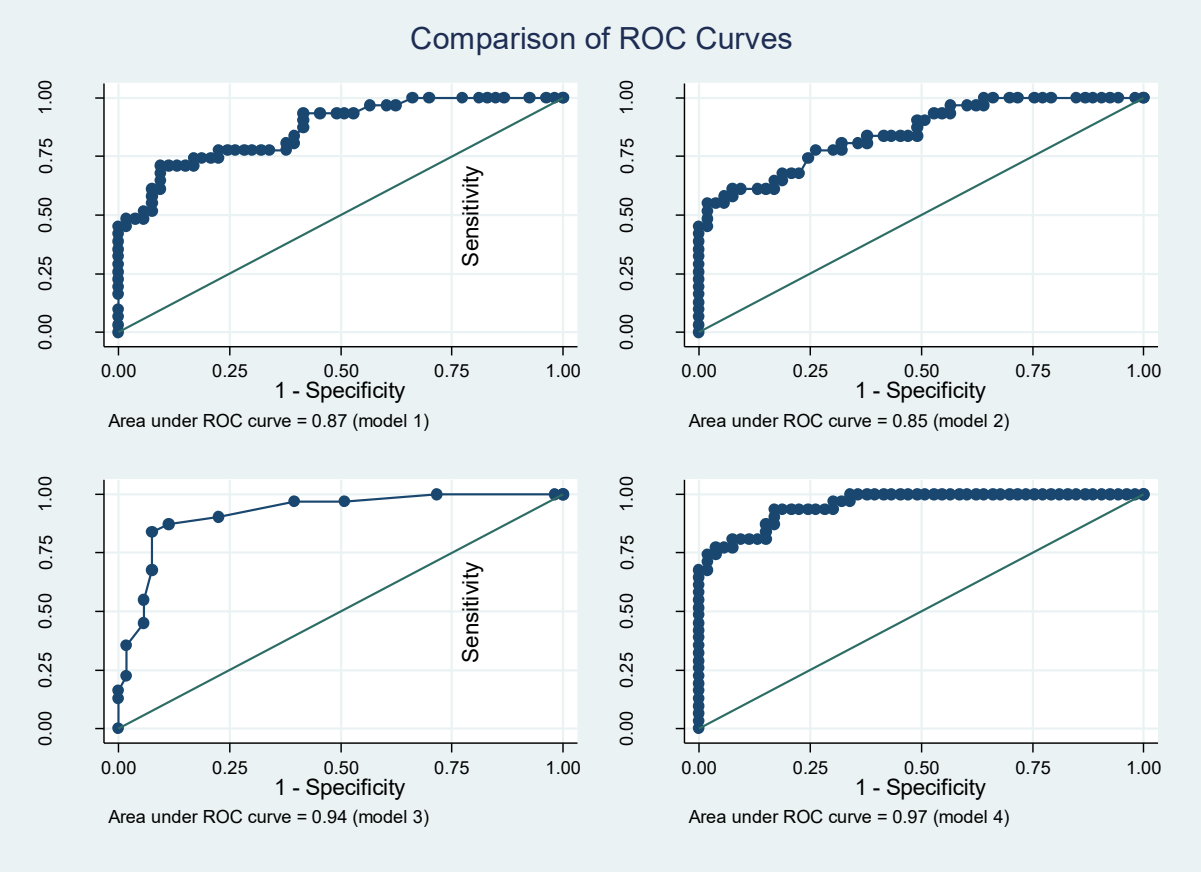


Figure 1. **ROC curves comparison of predictive models for ovarian malignancy**

4. Discussion

The purpose of the present study was to evaluate and compare the predictive value of a symptom-based score, CA125, and HE4 for the diagnosis of ovarian cancer in Iranian women with adnexal masses. The key findings demonstrated that while the combination of all three markers achieved the highest overall discriminative power (AUC: 0.971), the symptom-based score alone exhibited superior individual predictive performance with the most balanced diagnostic profile, featuring 83.87% sensitivity, 92.45% specificity, and 89.29% accuracy. Notably, in multivariate analysis, only the symptom score remained an independent significant predictor of malignancy, whereas CA125 and HE4 did not retain statistical significance.

The findings of the present study regarding the superior performance of the symptom score alone and in combination with CA125 and HE4 are aligned with the results of the studies by other research **(16,18)**. The study by Krishnamurthy et al. demonstrated that a symptom score (with a cut-off of≥9) had acceptable

sensitivity and specificity (84.8% and 88.6%), and its combination with CA125 led to a significant improvement in sensitivity (96.4%) and negative predictive value (97.4%) **(18)**. This consistency confirms the predictive power of symptom-based systems across different populations. Similarly, the Andersen et al. study **(16)** indicated that combining the Symptom Index (SI) with CA125 and HE4 (using a "2 out of 3 positive tests" rule) achieved a high sensitivity of 84% and a specificity of 98.5%. This supports our finding that a multimodal approach incorporating symptoms and biomarkers yields the highest overall discriminatory power.

However, our findings are partially misaligned with those of Gao et al., (2022) **(15)** and Aziz et al., (2023) **(19)**, highlighting potential population-specific and methodological differences. The study by Gao et al. constructed a nomogram for predicting ovarian malignancy, identifying menopausal status, BMI, HE4, and CA125 as significant predictors **(15)**. In our multivariate analysis, however, only the symptom score remained an independent predictor, CA125 and HE4 did not. This discrepancy may be attributed to differences in the studied populations, the composition of the patient cohorts, or the specific symptom scoring systems used. Their study included a broader range of clinical variables, which may have diluted the independent effect of biomarkers in our more focused model.

Furthermore, our results stand in clear contrast to those of Aziz et al., (2023) conducted on Pakistani population, which reported that the symptom index showed very low sensitivity (31.43%) and modest specificity (79.29%), leading the authors to conclude its limited utility as a standalone screening tool. In contrast, our symptom score demonstrated high sensitivity (83.87%) and specificity (92.45%). This stark difference likely stems from variations in the specific symptoms included in the scoring systems, cultural differences in symptom perception and reporting, and potentially differences in disease stage at presentation between the two study populations. This underscores that while symptom assessment is powerful, the specific operationalization of the symptom score and the context in which it is applied are critical determinants of its diagnostic performance.

This study has some limitations. First, the relatively small sample size may limit the statistical power and generalizability of the findings. Second, the single-center design conducted at a tertiary referral hospital in Tehran may introduce selection bias, as the patient population might not be fully representative of the broader Iranian community or primary care settings. Third, the symptom score, while practical, is subjective and could be influenced by patient recall bias or variability in symptom reporting. Finally, the study included only patients with O-RADS 4 masses scheduled for surgery, which is a high-risk preselected group; therefore, the results may not be applicable to women with lower-risk adnexal findings or to asymptomatic screening populations. To address the aforementioned limitations, future studies with larger sample sizes and a multicenter design across different levels of healthcare delivery are recommended to enhance the generalizability of the findings. Furthermore, it is suggested that the symptom-based score be validated in lower-risk populations using standardized tools and prospective daily symptom recording (e.g., mobile health applications) to reduce recall bias and better elucidate the true role of this score in the early detection and screening of ovarian cancer.

5. Conclusion

As evidenced by the results of the present study, a simple symptom-based scoring system is a highly effective tool for predicting ovarian malignancy in Iranian women presenting with adnexal masses, outperforming the individual biomarkers CA125 and HE4 in terms of balanced diagnostic accuracy and independent predictive value. Although the combination of the symptom score with both biomarkers yielded the highest discriminative ability, the symptom score alone provided an optimal balance of sensitivity and specificity for clinical use. These findings highlight the significant role of systematic symptom assessment in the diagnostic triage of adnexal masses and suggest that incorporating such a score into clinical practice could enhance early detection strategies for ovarian cancer in similar populations.

6. Declarations

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Ethical Considerations

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Ethics Code: IR.SBMU.RETECH.REC.1404.654). All participants provided informed written consent prior to enrollment.

Authors' Contributions

M.V and M.S.M contributed to conceptualization. M.S.M and M.S.H performed methodology and wrote the original draft. F.F and M.T participated in formal analysis: Farah Farzaneh, Maryam Talayeh. M.V and M.A helped in writing- review and editing and supervision of the study process. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Conflict of Interest

The authors declared no conflict of interest regarding the publication of this paper.

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