European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 9, 2020

The Role of HE4 in diagnosis and preoperative staging of epithelial ovarian cancer

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ABSTRACT

BACKGROUND: *The goal was to evaluate human epididymis protein 4 (HE4) in epithelial ovarian cancer diagnosis and preoperative staging.*

PATIENTS AND METHODS: *All females presented in the period from 1/3/2018 to 1/3/2019 to the National Cancer Institute -Cairo University with an adnexal mass and had initial serum tumour marker levels and pathology documented. We collected Patients' data from hospital records. Statistical analysis and data correlations were done to compare HE4 with pathological type and stage and with other markers.*

RESULTS: *We included 111 females. Forty-five patients were diagnosed with EOC. Postmenopausal HE4 was statistically significant, with the area under curve (AUC) 0.711 and p-value 0.005.HE4 showed a sensitivity of 68.2% and specificity of 60 %, PPV of 83.3%, NPV of 56.3%, and total accuracy of 70.6% by using a cut-off value of 64.6 Pmol/L. For premenopausal women, HE4 was statistically insignificant. Higher levels of preoperative HE4 were associated with advanced stages of EOC (III, IV) more than early stages.*

CONCLUSION: *HE4 represents a promising marker of EOC. It does not show high levels in benign conditions. Preliminary results indicate that HE4 can play a role in the diagnosis and preoperative staging of EOC.*

KEYWORDS: *Epithelial ovarian cancer, CA125, HE4, Risk of ovarian malignancy algorithm (ROMA), Ovarian tumour markers*

INTRODUCTION

Ovarian cancer in women is a highly lethal disease with age-standardized death rates of 3.9 per 100,000 women..[1]

Early detection of the disease significantly affects the survival of patients.[2],[3] Unfortunately, many patients are diagnosed at an advanced stage because it usually presents with no or little signs and symptoms in early stages.[4] The most commonly utilized tumour marker for ovarian cancer is cancer antigen 125(CA125). However, CA125 is limited in sensitivity. Its level is elevated in about 85% vs 50% in advanced and early stages of epithelial ovarian cancer (EOC), respectively. Moreover, in benign gynecologic conditions like uterine myomas, endometriosis, and pelvic inflammatory disease, it can be increased. therefore, the specificity of CA125 decreases to 75%.[5] Great efforts had been made to identify a novel marker for ovarian cancer with high sensitivity and specificity to enhance earlier stage diagnosis.[6]The human epididymis protein 4 (HE4) was found to be over-produced in ovarian cancer in 1999.[6] HE4 was first considered to be a likely serum biomarker for ovarian cancer in 2003.[7] Afterwards, Moore et al. applied a mixture of CA125HE4and menopausal status in many papers to detect ovarian cancer preoperatively. [6],[8] The benefit of HE4 over CA125 has been its reduced expression of healthy ovarian tissue. Whereas CA125 levels in premenopausal women with benign ovarian tumors are frequently increased and non-neoplastic conditions, serum levels of HE4 remain normal.[7],[9] Also HE4 levels in malignancies other than EOC are much less elevated compared to CA125.[10] However, high levels of HE4 are found in non-malignant diseases as renal failure[11] and heart faliure[12] and also in malignancies other than EOC as endometrial cancer, urothelial carcinoma, and lung adenocarcinoma.[10],[11]

AIM OF THE WORK

1- To evaluate The Role of serum HE4 in the diagnosis of EOC.
2- To evaluate The Role of serum HE4 in preoperative staging of EOC and hence preoperative surgical decision making.

PATIENTS AND METHODS

This is a retrospective diagnostic accuracy research that includes all female patients submitted to the Cairo University National Cancer Institute with an adnexal mass, over one year from1/3/2018 to 1/3/2019.

IRB approval: IRB00004025

***Inclusion criteria:** All females presented with adnexal mass and had:

- Pathology type documented (weather underwent upfront surgical exploration or biopsy taken for initial diagnosis before starting chemotherapy).
- AND initial serum HE4 and CA125 documented.

*** Exclusion criteria:** Any female presented with adnexal mass with:

- Initial pathology not documented.
- OR initial serum HE4 or CA125 not documented.

Medical records:

Data from the hospital-based registry of the National Cancer Institute has been used to create a list of all females included. Records were scrutinised at archives of Surgery, Clinical Pathology, Radiology, and Pathology departments. All pathological specimens were re-examined and revised.

Data:

We used A pre-determined sheet to fulfil the objectives of the study. We reviewed all patients' files to obtain all available data as follows:

- Name of the Patient
- Age of the Patient
- Menopausal status
- Radiological investigations: Pelviabdominal ultrasound, CT scan, or MRI.
- Radiological TNM Staging
- Tumour markers: preoperative HE4 and CA125.
- Histological type (Biopsy OR Surgical Specimen)
- Presence of tumour deposits in the omentum, appendix, pelvic lymph nodes, or peritoneum (intraoperative staging, if possible).
- Pathological TNM staging (if possible)

Data analysis and correlations:

Correlation analysis was done comparing:

1- HE4 results with the pathological results.

2- HE4 values relative to tumour staging (pathological, radiological, and intraoperative).

Simple comparisons were made between specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV), and total preoperative HE4, CA125 accuracy and risk of ovarian malignancy algorithm (ROMA)score for EOC diagnosis and staging.

Statistical methods:

IBM SPSS Advanced Statistics (Statistical Package for Social Sciences), version 24, was used to analyse the data (SPSS Inc., Chicago, IL). We expressed numerical data as median and range or mean and standard deviation as it fits, Whereas the number and percentage were represented as qualitative data. As suitable, we used the Chi-square (Fisher's exact) test to examine the relationship among qualitative variables.

To estimate the best cut-off point, we used Receiver Operating Characteristics (ROC) curve. Then calculation of sensitivity, specificity with their 95% confidence interval was done between EOC cases and non-EOC cases. Correlation analysis between the marker levels and other covariates using Pearson correlation was performed.

A p-value equal to or less than 0.05 was deemed to be statistically significant. All of the tests were two-tailed.

Calculation of ROMA as by Moore et al. .[7]:

- A predictive index (PI):

Premenopausal: PI = −12.0 + 2.38 * LN [HE4] + 0.0626 * LN [CA125]

Postmenopausal: PI = −8.09 + 1.04 * LN [HE4] + 0.732 * LN [CA125]

LN = natural logarithm.

- ROMA value:

ROMA value (%) = exp(PI)/[1 + exp(PI)]*100 where, exp(PI) = ePI

RESULTS

The study included 111 female patients presented with an adnexal mass. Patient demographics and characteristics have been illustrated in (TABLE 1TABLE 1).

Patients have a mean age of 47.4 years, varying from 14 to 76 years, including 64 premenopausal women (57.7%) and 47 postmenopausal women (42.3%).

Preoperative radiological staging is illustrated in (TABLE 1). Ninety-one cases (82%) underwent upfront surgical exploration. The assessed intraoperative stage of these 91 patients is shown in (TABLE 1).

The pathological type of the 111 cases revealed that 45 patients were diagnosed with EOC. Eleven patients were diagnosed with non-EOC (six granulosa cell tumors and five mature cystic teratoma of the ovary). Twelve patients had borderline malignant tumours, and 24 patients had benign ovarian masses. There were 19 patients with non-ovarian origin (7 cases endometrial adenocarcinoma, 6 cases uterine leiomyoma, 4 inflammatory cases, and two cases TCC of the urinary bladder).

TABLE 1. Patients' and tumors' characteristics (n=111)

Characteristic	Frequency	Percentage
Menopausal status		
Premenopausal	64	57.7
Postmenopausal	47	42.3
1ry Tumor site		
Ovarian	92	82.8
Extra ovarian	19	17.2
Microscopic picture		
Malignant	77	69.4
Non-Malignant	34	30.6
I II III	69	62.2
IV	14	12.6
	22	19.8
	6	5.4
Upfront Surgery Yes	91	82
No	20	18
Intraoperative stage (n=91)		
I II III IV	62	68.1
	10	11
	17	18.7
	2	2.2
Histological grade (n=32)		
I II III	4	12.5
	14	43.75
	14	43.75
Pathological staging (n=80)		
I II III IV	47	58.7
	3	3.8
	24	30
	6	7.5

Pathological staging was possible to obtain in 80 cases and was as follows: 47 stage I patients, three stage II patients, 24 stage III patients, and six stage IV patients, respectively.

Using the ROC analysis and the area under curve (AUC) measurement for each marker, the diagnostic precision of serum CA 125, HE4 and ROMA in distinguishing EOC from other adnexal masses was demonstrated as shown in (FIGURE1 & FIGURE2).

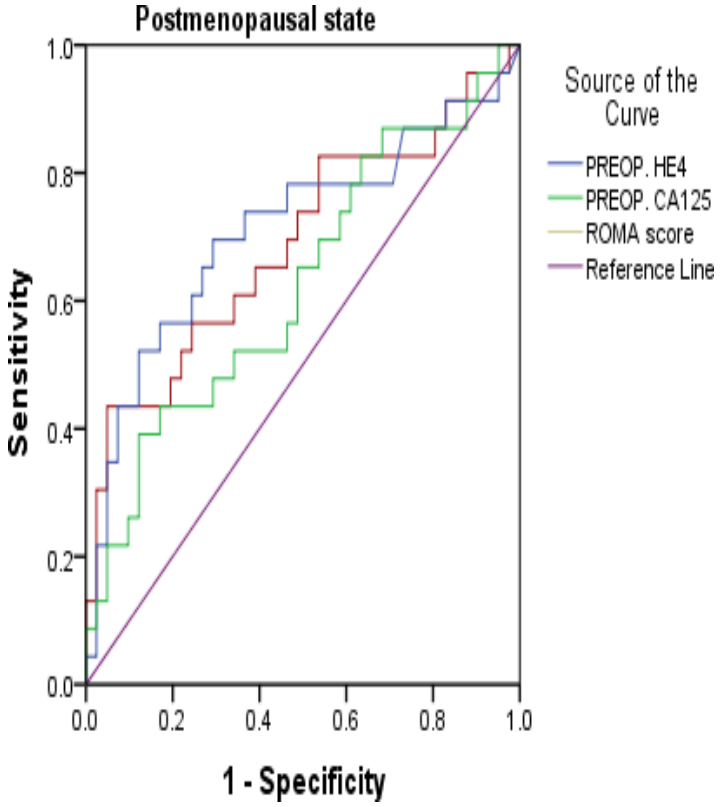

FIGURE1. Receiving Operating Characteristic (ROC) curve of HE4, CA125 and ROMA score for postmenopausal patients
Abbreviations:HE4: Human Epididymis Protein 4, CA125: Cancer Antigen 125, ROMA: Risk of Ovarian Malignancy Algorithm, PREOP: preoperative

Postmenopausal HE4 and ROMA score were statistically significant with AUC (0.711 VS 0.689) and p-value (0.005 vs 0.013), while CA 125 was statistically insignificant with AUC 0.626 and p-value 0.097. For premenopausal women, HE4, CA125, and ROMA score were statistically insignificant with AUC for HE4 still more than ROMA score (0.609 vs 0.584) and both more than CA125 (0.433) as illustrated in (TABLE 2).

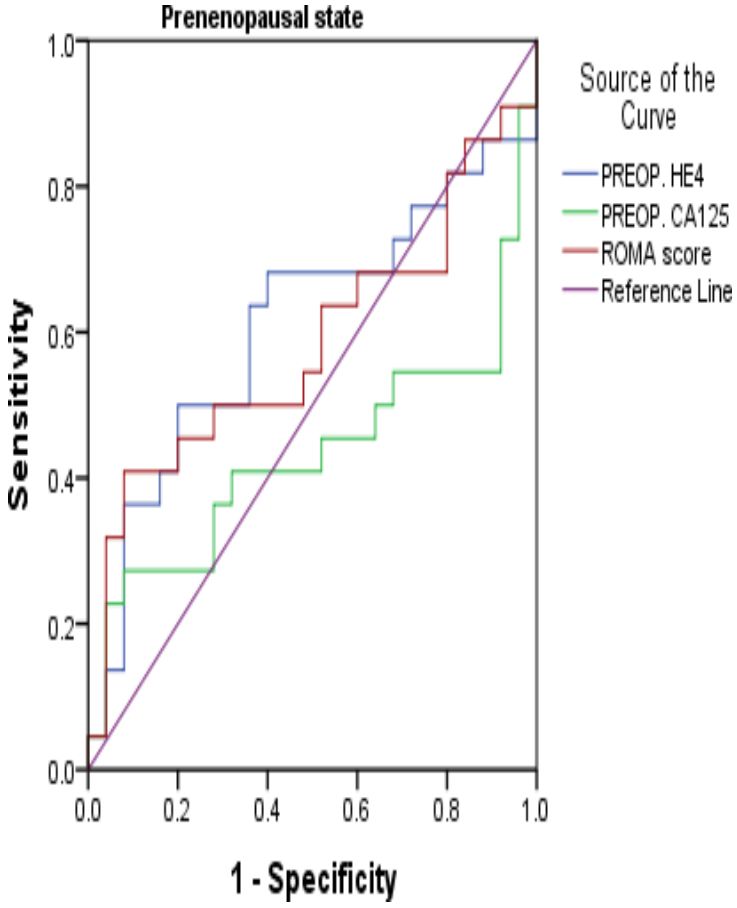

FIGURE2. Receiving Operating Characteristic (ROC) curve of HE4, CA125 and ROMA score for premenopausal patients
Abbreviations:HE4: Human Epididymis Protein 4, CA125: Cancer Antigen 125, ROMA: Risk of Ovarian Malignancy Algorithm, PREOP: preoperative

For each HE4, ROMA score, and CA125, we considered two cut-off values. One in premenopausal patients, and one in postmenopausal patients. We obtained the best sensitivity and specificity for each marker at the proposed cut-off value for each menopausal status. We also identified the PPV, NPV, and total accuracy, as shown in (TABLE 2).

TABLE 2. Receiving operating characteristics (ROC) curve analysis and AUC of preoperative HE4, ROMA score & CA125

Marker/Score & menopausal status	cut- of point	Sensitivity	Specificity	PPV	NPV	Total accuracy	AUC	P-value

HE4								
postmenopausal	59.5	69.6%	71.0%	66.7%	68.2%	67.4%	0.711	0.005
Premenopausal	64.6	68.2%	60.0%	83.3%	56.3%	70.6%	0.609	0.0.201
ROMA score								
Postmenopausal	18.7	60.9 %	66.0%	58.3%	59%	58.7%	0.689	0.013
Premenopausal	21.1	54.5%	52%	70.1%	41.2%	55.9%	0.584	0.327
CA125								
postmenopausal	47.9	52.2%	54.0%	46.1%	45%	45.7%	0.626	0.097
Premenopausal	32.9	45.5%	48%	62.5%	33.3%	47%	0.433	0.430

Abbreviations: HE4: Human Epididymis Protein 4, ROMA: Risk of Ovarian Malignancy Algorithm, CA125: Cancer Antigen 125, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: area under the curve

Univariate analysis was done to study the factors more to be associated with EOC vs other pathologies revealed that the preoperative HE4 level, ROMA score were statistically significant and associated with EOC with a p-value of (<0.001, 0.031) respectively. CA125 level was not significant statistically, as shown in (TABLE 3) Applying multivariate analysis by logistic regression model to preclude the effect of confounding factors that present on the univariate level and to indicate the only independent predictive factors revealed that the only factor strongly and significantly associated with EOC was the level of preoperative HE4.

TABLE 3. Predictors of Epithelial Ovarian Cancer (EOC)

Variable	**Preoperative level**	**(n= 111)**	**Histopathology**		**p-value**
			EOC (n=45)	**Others (n=66)**	
Preoperative HE4	**[*a]<=cutoff**	**No**	14	44	<0.001
		%	31.1%	66.7%	
	[*a]> cut-off	**No**	31	22	
		%	68.9%	33.3%	
Preoperative CA125	**[*b]<=cutoff**	No	23	34	0.967
		%	51.1%	51.5%	
		No	22	32	

	***b> cut-off**	%	48.9%	48.5%	
Preoperative ROMA score	***c low risk**	No	20	43	0.031
		%	44.4%	65.2%	
	***c high risk**	No	25	23	
		%	55.6%	34.8%	

Abbreviations: EOC: Epithelial Ovarian Cancer, HE4: Human Epididymis Protein 4, CA125: Cancer Antigen 125, ROMA: Risk of Ovarian Malignancy Algorithm
***a:** cut off value of HE4 for premenopausal (64.6) Pmol/L & for postmenopausal (59.5) Pmol/L.
***b:** cut off value of CA125 for premenopausal (32.9) U/ml & for postmenopausal (47.9) U/ml.
***c:** cut off value of ROMA score for premenopausal (21.1%) & for postmenopausal (18.7%).

Thirty-one cases with EOC were correleted with a high level of HE4 compared to 22 cases diagnosed as non-EOC (68.9% VS 33.3%) with p-value 0.031, as evident in (TABLE 3). This was statistically significant with the risk of cases of EOC to be with an up-regulated level of HE4 more than five times compared to other cases with p-value <0.001, Odds Ratio (OR) 4.8, 95% Confidence Interval (CI) for OR ranging from 2.1 to 11.1.

The increased levels of each HE4, CA125, and ROMA score were more correlated with the advanced stage of EOC (III, IV) than early ones (I, II). For HE4: 62.5% versus 12.5%, with a statistically significant p-value (<0.001), as in (TABLE 4). For ROMA score: 54.1% versus 23.3% respectively with statistically significant p-value = 0.005, evident in (TABLE 5). For CA125: 50.0% versus 27.3% respectively with statistically significant p-value =0.037 as shown in (TABLE 6).

TABLE 4. Association between preoperative HE4 and pathological staging

Pathological staging	**(n= 80)**	**Preoperative HE4**		**Total**	**P-value**
		<=cutoff *[a]	**>cutoff [*a]**		
Early-stage (I, II)	no	35	15	50	<0.001
	%	87.5%	37.5%	62.5%	
Late-stage (III, IV)	no	5	25	30	
	%	12.5%	62.5%	37.5%	
Total	no	40	40	80	
	%	100.0%	100.0%	100.0%	

Abbreviations : HE4: Human Epididymis Protein 4
***a:** cut off value of HE4 for premenopausal (64.6) Pmol/L & for postmenopausal (59.5) Pmol/L.

TABLE 5. Association between preoperative ROMA score and pathological staging

Pathological staging	(n= 80)	ROMA score		Total	P-value
		≤ cutoff *[a]	>cutoff *[a]		
Early-stage (I, II)	no	33	17	50	0.005
	%	76.7%	45.9%	62.5%	
Late-stage (III, IV)	no	10	20	30	
	%	23.3%	54.1%	37.5%	
Total	no	43	37	80	
	%	100.0%	100.0%	100.0%	

Abbreviations: ROMA: Risk of Ovarian Malignancy Algorithm
***a:** cut off value of ROMA score for premenopausal (21.1%) & for postmenopausal (18.7%).

TABLE 6. Association between preoperative CA125 and pathological staging

Pathological staging	(n= 80)	PREOP. CA125		Total	P-value
		<=cutoff *[a]	>cutoff *[a]		
Early-stage (I, II)	no	32	18	50	0.037
	%	72.7%	50.0%	62.5%	
Late-stage (III, IV)	no	12	18	30	
	%	27.3%	50.0%	37.5%	
Total	no	44	36	80	
	%	100.0%	100.0%	100.0%	

Abbreviations: CA125: Cancer Antigen 125
***a:**cut off value of CA125 for premenopausal (32.9) U/ml & for postmenopausal (47.9) U/ml.

DISCUSSION

Several studies tried to identify preoperative malignant ovarian masses.[13,14] We assessed the diagnostic efficiency of preoperative HE4, CA125 serum levels, and ROMA score using ROC curve analysis for predicting the possibility of EOC in female patients presented with ovarian masses.

In most papers, the cut-off values set by the producer for CA 125 are 35 U/mL, 140 pmol/L for HE4 and 11.4 % (premenopausal) and 29.9 %

(postmenopausal) for ROMA to assess the efficiency of each marker in the pelvic mass assessment.[15]

The sensitivity and specificity of 87.5 % and 75 % and 0.92 AUC for HE4 were stated by Sandri et al .[16] In our research, however, for premenopausal women, the best HE4 sensitivity was 68.2% and specificity of 60 % with 0.6 AUC by using a 64.6 Pmol/L cut-off value. The best sensitivity became 69.6 % for postmenopausal women, while the specificity became 71 % with 0.71 AUC using a cut-off value of 59.5 Pmol/L.

The ROMA cut-off value in our study was higher than that documented by Moore et al. [17] but lower than those stated by Anton et al. and Van Gorp et al., which were 39.68% and 35.9% for postmenopausal women, respectively. [13],[18]

Such different outcomes can be attributed to the different classifications of tumour types described in the discrete studies. Besides that, we aimed mainly at EOC, while other studies had great heterogenecity in histopathological tumour types. Such variations could also have arisen from the use of various CA125 kits.[15]

Our results showed that HE4 per se had a higher specificity than CA 125 for forecasting ovarian cancer in premenopausal ladies. These results were coherent with other studies.[13],[19],[20] CA125 may show very high levels in patients with benign ovarian conditions, unlike HE4.[9],[16],[20],[21],[22]. This may clarify the limited specificity of ROMA relative to HE4 in our research, as CA125 is integrated into the ROMA algorithm.

Previous studies clarified contradictory findings. Van Gorp et al. and Chan et al. recorded that in the detection of ovarian cancer in postmenopausal Caucasian and Asian women, ROMA and HE4 were comparable to CA 125, respectively.[18],[19] By comparison, in postmenopausal ladies, Sandri et al. and Montagnana et al. displayed that ROMA showed superb diagnostic execution.[16],[23]

In our study for postmenopausal women, serum HE4 and ROMA score were considerably superior to CA 125 in discriminating ovarian cancer. The ROMA sensitivity, however, was higher than CA 125, and both were less than HE4.

Bandiera et al. demonstrated that higher tumor marker levels and ROMA were correlated with advanced FIGO stage in EOC patients.[24] Liet al. revealed that high ROMA score was associated with advanced ovarian cancer and the best indicator of disease stage was ROMA. [25]

In our study, we found similar results that increased preoperative HE4, CA125 and high-risk ROMA score levels are more correlated with the advanced EOC stage (III, IV) than with the early stage (I, II).

The association of higher HE4 levels and advanced tumour stages means that HE4 may have a role in preoperative surgical decision making. For example, to decide whether to start with upfront surgery or chemotherapy, whether we will need a frozen section intraoperatively, and whether complete resection is possible or not. Actually, we need a much higher number of patients to identify such a cut-off level that guides the preoperative decision making.

In conclusion: HE4 represents a promising early detection method of EOC. It showed high diagnostic performance in EOC. It does not show high levels in benign conditions, compared to the more established biomarker CA 125. Preliminary results indicate that HE4 could play a role in preoperative staging and diagnosis, and hence surgical decision-making of EOC.

ACKNOWLEDGMENT

This article was concluded from an approved thesis presented by the third author to obtain the Master degree in surgical oncology. The first two authors are supervisors of the thesis. Other co-authors participated in the article preparation, editing, and reviewing.

Sincere appreciation and gratitude go to **Prof. Mohamed Al-Zohairy**, the primary supervisor of the thesis, for his continuous support, for his patience and immense knowledge. Unfortunately, he is not of the co-authors of this article, but he approved us for publishing it.

Mohammad Fouad Taher **(Mohammad, F., Taher)**
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AUTHOR CONTRIBUTIONS:

Mohammad Taher: thesis conceptualisation, literature research, data analysis, thesis supervision & revision, manuscript preparation, editing and reviewing.
Iman Abdelgawad: Laboratory data collection and analysis, thesis supervision, and manuscript reviewing.
Mohamed Fawzy: data collection and analysis, literature research, statistical analysis, thesis preparation and discussion and manuscript reviewing.
Rasha Allam: data collection, statistical analysis and correlations, thesis revision and manuscript editing and reviewing.
Ahmed Lymona: data collection and analysis, literature research, and manuscript reviewing.

Maher Ibraheem: data analysis, literature research, manuscript conceptualisation, preparation, and reviewing.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE:

This study was a retrospective study based on a review of medical records of the included patients. Approval of National Cancer Institute – Cairo University ethical committee was secured through the expedited pathway. All measures were taken to protect the security and confidentiality of the individual patient's information. All procedures performed in the study involving human participants were in accordance with the Declaration of Helsinki.

CONSENT TO PUBLISH:

All participants have provided acceptance and consents for publication of their details. All personal information has been made anonymous.

DATA AVAILABILITY:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST: No conflicts of interest.

FUNDING SUPPORT:

Apart from the ordinary support of the National Cancer Institute – Cairo University in serving all patients freely as a University Hospital, no extra funds were needed.

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin D. M, Piñeros M. et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. **144**, 1941-1953 (2019).
2. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. *Arch Gynecol Obstet*. **290**, 839-842 (2014).

3. Rauh-Hain J, Krivak T, Marcela G del C, Alexander B. Ovarian cancer screening and early detection in the general population. *Rev Obstet Gynecol*. **4**, 15-21 (2011).
4. Lalwani N, Prasad SR, Vikram R, Shanbhogue AK, Huettner PC, Fasih N. Histologic, molecular, and cytogenetic features of ovarian cancers: Implications for diagnosis and treatment. *Radiographics*. **31**, 625-646 (2011).
5. Jacobs I, Bast R. The CA 125 tumour-associated antigen: a review of the literature. *Hum Reprod*. **4**, 1-12 (1989).
6. Moore RG, Miller MC, Disilvestro P, Lisa M. G, Walter B, John J.S et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. *Obstet Gynecol*. **118**, 280-288 (2011).
7. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ et al. A novel multiple marker bioassay utilising HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol*. **112**, 40-46 (2009).
8. Heravi-Moussavi A, Anglesio MS, Cheng SWG, Grace S, Janine Y, Winnie P et al. Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers. *N Engl J Med*. **366**, 234-242 (2012).
9. Huhtinen K, Suvitie P, Hiissa J, Junnila J, Huvila J, Kujari H et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br J Cancer*. **100**, 1315-1319 (2009).
10. Karlsen NS, Karlsen MA, Høgdall CK, Høgdall EVS. HE4 Tissue Expression and Serum HE4 Levels in Healthy Individuals and Patients with Benign or Malignant Tumors: A Systematic Review. *Cancer Epidemiology Biomarkers Prev*. **23**, 2285-2295 (2014).
11. Molina R, Escudero JM, Augé JM, Xavier F, Laura T, Aureli L et al. HE4 a novel tumour marker for ovarian cancer: comparison with CA 125 and ROMA algorithm in patients with gynaecological diseases. *Tumour Biol*. **32**, 1087-1095 (2011).
12. Piek A, Meijers W, Schorten N, Gansevoort NF, de Boer RT, Sillje RA. HE4 serum levels are associated with heart failure severity in patients with chronic heart failure. *J Card Faliure*. **23**, 12-19 (2017).
13. Anton C, Carvalho FM, Oliveira EI, Maciel GAR, Baracat EC, Carvalho JP. A comparison of CA125, HE4, risk ovarian malignancy algorithm (ROMA), and risk malignancy index (RMI) for the classification of ovarian masses. *Clinics*. **67**, 437-441 (2012).
14. Ulusoy S, Akbayir O, Numanoglu C, Ulusoy N, Odabas E, Gulkilik A. The risk of malignancy index in discrimination of adnexal masses. *Int J Gynecol Obstet*. **96**, 186-191 (2007).
15. Bouzari Z, Rahimi H, Hemmat G, Shahla Y, Karimollah H-T, Mohammad J. Cancer Antigen 125 (CA125), Human Epididymis Protein 4 (HE4), Risk of Malignancy Index (RMI), and Risk of Ovarian Malignancy Algorithm (ROMA) as Diagnostic Tests in Ovarian Cancer. *Int J Cancer Manag*. **12**, e59395 (2019).
16. Sandri M, Bottari F, Franchi D, Boveri S, Candiani M, Ronzoni S et al. Comparison of HE4, CA125 and ROMA algorithm in women with a pelvic mass: correlation with pathological outcome. *Gynecol Oncol*. **128**, 233-238 (2013).
17. Moore R, Jabre-Raughley M, Brown A, Robison KM, Miller MC, Allard WJ et al. Comparison of a novel multiple marker assay vs the Risk of Malignancy Index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. *Am J Obstet Gynecol*. **203**, 228.e1-228.e6 (2010).
18. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F et al. HE4 and CA125 as a diagnostic test in ovarian cancer: Prospective validation of the Risk of Ovarian Malignancy Algorithm. *Br J Cancer*. **104**, 863-870 (2011).
19. Chan K, Chen C, Joo H, Ochiai K, Wilailak S, Choon AT et al. The use of HE4 in the prediction of ovarian cancer in Asian women with a pelvic mass. *Gynecol Oncol*. **128**, 239-244 (2013).
20. Holcomb K, Vucetic Z, Miller M, Robert C. Human epididymis protein 4 offers superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women. *Am J Obs Gynecol*. **205**, 358.e1-358.e6 (2011).
21. Chung SH, Lee SY, Ju W, Kim SC. Clinical efficacy of serum human epididymis protein 4 as a diagnostic biomarker of ovarian cancer: A pilot study. *Obstet Gynecol Sci*. **56**, 234 (2013).

22. Wang S, Dong L, Li H, Wang M. The Application of HE4 in Diagnosis of Gynecological Pelvic Malignant Tumor. *Clin Oncol Cancer Res*. **6**, 72-74 (2009).
23. Montagnana M, Danese E, Ruzzenente O, Valentina N, Teresita G, Matteo S et al. The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in women presenting with pelvic mass: Is it really useful? *Clin Chem Lab Med*. **49**, 521-525 (2011).
24. Bandiera E, Romani C, Specchia C, Zanotti C, Galli L, Claudio Ruggeri C, et al. Serum Human Epididymis Protein 4 and Risk for Ovarian Malignancy Algorithm as New Diagnostic and Prognostic Tools for Epithelial Ovarian Cancer Management. *Cancer Epidemiol Biomarkers Prev*. **20**, 2496-2506 (2011).
25. Li QL, Wang CJ, Qi P, Zhang YX. Correlation of preoperative ROMA scores with clinical stage in epithelial ovarian cancer patients. *Clin Transl Oncol*. **19**, 1260-1267 (2017).