Research Article

Determining the Diagnostic Strength of Serum Tumor Markers in Epithelial Ovarian Cancer in Women from Lahore, Pakistan

Rizwana Hussain,[1] Saba Khaliq,[2] Hafiz Usman Ahmad[3]

[1]Department of Physiology, Allama Iqbal Medical College, Lahore, Pakistan; [2]Department of Physiology, University of Health Sciences, Lahore, Pakistan; [3]Lab Technology Department, University of Health Sciences, Lahore, Pakistan

Abstract

Background: Ovarian cancer is tagged as deadly condition worldwide. The delay in diagnose is due to late presentation and lack of specific screening tests. HE4 and CA15-3 are considered as noninvasive marker with varied cut off reference and sensitivity, specificity among various populations.

Objective: The objective of this study was to investigate correlation along with sensitivity, specificity, PPV, NPV of HE4, CA15-3 and CA125 among epithelial ovarian cancer.

Methods: This cross section comparative study was conducted on 70 diagnosed ovarian cancer cases age 20 to ≥70 year of any FIGO stage I-IV after approval from IRB and ethical review board (UHS/Reg-17/ERC-4659) of University of Health Sciences. The blood samples were taken and serum levels of HE4 and CA15-3 were measured by ELISA. While CA125 was determined from patient investigation reports.

Results: This study found positive correlation (r=0.089 p=0.66) between malignant and borderline ovarian tumors for HE4, while negative correlation between early (I and II) and advanced (stage III and IV) FIGO stage with r=-0.028 p=0.876. Whereas, for CA15-3 negative correlation was observed with r= -0.006 p=0.980 between malignant and borderline variants of epithelial ovarian cancer and also negative correlation was seen between early (I and II) and advanced (stage III and IV) FIGO stage with r= -0.095 p=0.588.

Conclusions: The discriminative strength of HE4 as biomarker determined by ROC curve was 0.55%. Hence HE4 can be used for diagnosis or prognosis of ovarian cancer.

Received: 18-10-2023 | **Revision:** 22-05-2024 | **Accepted:** 19-08-2024

Corresponding Author | Dr. Rizwana Hussain, Associate Professor of Physiology, Allama Iqbal Medical College, Lahore, Pakistan **Email:** rizwanahussain2579@gmail.com

Keywords | CA125, CA15-3, Correlation, FIGO, HE4, Ovarian Cancer

Introduction

Ovarian cancer is most lethal malignancy because of its presentation is usually at advanced stage. This is also considered as silent killer.[1] According to world health organization eight histological subtypes of tumor are described, which are serous, mucinous, endometrioid, clear cell, transitional cell, squamous cell, mixed epithelial and undifferentiated. Among all these they have been further labelled as benign, malignant or borderline. Borderline variant is one with less aggressive.[2] The researchers revealed role of genetic mutations and polymorphisms in ovarian malignancy. Many proto-oncogenes tumor suppressor genes were found altered and considered as root cause of tumor

Production and Hosting by KEMU

https://doi.org/10.21649/akemu.v30i3.5461

development.[3,4] The disease is presented usually in later stage with varied symptoms. To begin with women are screened with ultrasonography but ovarian mass cannot be described as benign or malignant just on basis of ultrasonography. The gynecologists therefore must seek help of certain serum markers such as CA125. The role of CA125 as well as other novel markers such as HE4, CA15-3, CCL18, VEGF is promising for diagnosis as well as prognosis. CA125 is not much reliable as its levels are raised only in 50% of early stage ovarian cancer.[5] Also, there are other gynecological and non-gynecological pathologies in which CA125 is elevated, so it is no more first investigation of choice. To reduce burden of high mortality linked with ovarian cancer it is essential to identify exclusive biomarkers in complement to CA125.[6] The unavailability of exclusive screening biomarkers for early detection of ovarian cancer generated the need of more reliable and efficient tools. In this scenario various biomarkers are already under evaluation by researchers, such as HE4 and CA15-3.

CA-125 was first discovered in 1981.[7,8] This is most commonly checked tumor marker and is used to monitor efficacy of treatment along with detection of recurrence. Elevated levels of CA125 are found in 47 % of early stage while 80%-90% raised levels are seen in advanced stage of ovarian cancer.[6] This marker has been utilized to monitor prognosis of diagnosed ovarian cancer women but its efficacy is increased many fold when assessed in combination with new markers. The prognosis and survival in ovarian cancer is dependent on early diagnosis. So, to meet this goal HE4 and CA15-3 together or alone can be utilized to improve outcome by decreasing mortality.[9] Also literature review supports the utilization of CA125 for assessment of response to therapy and well follow up.[10]

He4 is protein which possess WAP type disulfide core and is encoded by WFDC2 gene which is located on chromosome 20q1213.1. It is found elevated in different subtypes of epithelial ovarian cancer.[6] HE4 was first found in epididymis of male.[11] Later, its presence was also revealed in epithelial tissue of ovaries.[12] The normal tissue expression is limited to epithelium of reproductive tract[13] but its levels are elevated in serum of epithelial ovarian cancer.[14] HE4 is proved to be better biomarker than CA125 while differentiating benign and borderline gynecological cancers including early stage of EOC.[6] Some researchers found raised levels of HE4 in various subtypes of EOC. Moreover, HE4 has clear specificity difference over CA125 also marked sensitivity for EOC (Epithelial Ovarian Cancer) especially in early stages.[15] Although, its correlation with CA125 is not well established as there were varied results. But even then combined use of these markers can be beneficial in certain subtypes of EOC. HE4 and CA125 were found correlated with serous tumor type and stage.[15] Another study by Alsomairi et al[16] revealed varied expression of serum markers in subtypes of ovarian cancer including HE4 and CA125 and CA15-3.

The discovery of CA15-3 is linked with breast cancer. In 1984 two murine monoclonal antibodies 115D8 and DF3 were prepared which were found to react with antigens on human breast cancer cells.[17] The 115D8 and DF3 reactive determinants were found located on high molecular weight glycoprotein called mucin1 or polymorphic epithelial mucin. It is encoded by MUC1 gene. This protein is expressed in apical lumen of glandular epithelium. In malignancy the polarization of this protein and gene is lost. So, mucin1 is shed from cancer cells and can be detected easily in serum in high concentrations.[18] It is a mucinous tumor marker and is elevated in certain tumors.[19] CA 15-3 consists of 3 domains and 2 subunits. The extracellular subunit contains 20 amino acid residues.[20] Earlier research unveiled the fact that elevated CA15-3 in advanced breast cancer later its raised levels were acknowledged in ovarian cancer too[21,22] with sensitivity 71% and specificity 95% In another research, the relationship between high levels of CA15-3 with disease recurrence as well as FIGO stage was studied.[23] Hence, this noninvasive marker was proven to be useful in the detection of tumor at early stage. However, none of the internationally accepted standard investigation panel like ROMA 1 included CA15-3 for screening adnexal mass. This limitation of unavailability of standard scale of diagnosis of ovarian cancer lead researchers to the conclusion to evaluate CA15-3 in combination with HE4 and conventional marker CA125 proved to be more promising.[24]

Methods

Ethical review board of University of Health Sciences Lahore (UHS/REG-17/ERC 4659) had given approval to conduct this study in accordance with Helsinki decla-

ration of human rights.[25] Study design was Cross sectional Comparative. The subjects were recruited from INMOL Hospital Lahore, Sheikh Zayed Hospital Lahore and Hijaz Hospital Lahore. After taking written informed consent the blood samples were collected. All experimental work was carried out in Department of Physiology and Cell Biology, University of Health Sciences, Lahore.

The sample size was calculated using the following formula

$$n = \frac{2\sigma^2(z_{1-\alpha/2} + z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

The sample size was also calculated by taking the mean levels of micro ribonucleic acid-93 (miR-93) in the same formula, with level of significance 5, power of test 95%, population standard deviation 0.5326, population variance 0.280926, test value of population mean 1.6814, anticipated population mean 2, and sample size 70.

Those included were females aged 20-70 years diagnosed with ovarian cancer and those tentatively planned for any of the following surgical procedures: unilateral or bilateral oophorectomy, salpingo-oophorectomy via laparotomy or laparoscopy, subtotal resection, or removal of tumour fragments, and hysterectomy with salpingo-oophorectomy. Patients already on chemotherapy were excluded

Blood sample (2.5 ml) was taken from diagnosed patients of Ovarian Cancer in green top tube. The tube was centrifuged for separation of plasma. Plasma was preserved in eppendorf for Elisa. The eppendorfs were labelled properly and stored at -20°C.

Serum HE4 was measured by human HE4 ELISA kit (Cat No.PRS-01719hu) manufactured by Glory Science). The ELISA kit was able to determine HE4 concentrations in human serum, blood plasma and other biological fluids. The ELISA kit was able to detect HE4 concentrations ranging from 2pmol/L-40pmol/L. The Kit uses double antibody sandwich enzyme-linked immunosorbent assay to determine the level of human HE4 in samples. The serum samples and standards are added to the wells which are pre-coated with human HE4 monoclonal antibody. Biotin conjugated antihuman HE4 antibody and HRP (horseradish peroxidase) are added, Biotin-conjugated antihuman HE4 antibody binds to human HE4 captured by the first antibody. Following incubation and first washing TMB (tetramethylbenzidine) substrate solution which is reactive to HRP is added to well. A colored product is formed in proportion to amount of human HE4 present in sample or standard. The reaction is terminated by adding sulphuric acid (stop solution). The absorbance is measured using an automated ELISA reader. The values of OD were obtained, and the actual concentration of the samples was calculated by multiplying the concentrations with the dilution factor[27].

Serum CA15-3 was measured by human CA15-3 ELISA kit (Cat No.PRS-00993hu) manufactured by Glory Science). The ELISA kit was able to determine CA15-3 concentrations in human serum, blood plasma and other biological fluids. The ELISA kit was able to detect Ca15-3 concentrations ranging from 7U/mL-2007U/Ml. The Kit uses double antibody sandwich enzyme-linked immunosorbent assay to determine the level of human CA15-3 in samples. The serum samples and standards are added to the wells which are pre-coated with human CA15-3 monoclonal antibody. Biotin conjugated antihuman CA15-3 antibody and HRP (horseradish peroxidase) are added, Biotin-conjugated antihuman CA15-3 antibody binds to human CA15-3 captured by the first antibody. Following incubation and first washing TMB (tetramethylbenzidine) substrate solution which is reactive to HRP is added to well. A colored product is formed in proportion to amount of human CA15-3 present in sample or standard. The reaction is terminated by adding sulphuric acid (stop solution). The absorbance is measured using an automated ELISA reader. The values of OD were obtained, and the actual concentration of the samples was calculated by multiplying the concentrations with the dilution factor.[26]

Results

The data was entered in Statistical package for Social Science Version 24 and analyzed. Frequency and percentages were calculated for qualitative variable and mean with standard deviation were calculated for quantitative variable. One-way ANOVA was applied to compare the groups in age, stage and tumor on the base of HE4, CA15-3 and CA125. The correlation test was applied to investigate the relationship between Malignant Vs

Borderline and Stage I, II Vs Stage III & IV on the base of HE4, CA125 and CA15-3. Sensitivity and specificity calculated between HE4, CA15-3 and CA125 follow up with Tumor, age, stage, parity and side. ROC curve were also made on base of HE4, CA125 and CA15-3 by considering tumor, age, stage, parity and side as grouping variable.

This study reported mean age 45.63 years (±11.98) of ovarian cancer women who participated in this research. The mean level of HE4 was found HE4=20.79 pmol/L (5.19pmol/L-268.5pmol/L). The mean CA125 Baseline =1126.34 U/mL and Follow up=77.0 U/mL was explored. While, the mean level of CA15-3 found in this study was, 51.50 U/mL (0- U/mL -796.2 U/

There is positive correlation of HE4 for malignant and borderline ovarian groups with insignificant p-value, reflecting that when cancer status change then HE4 gradually increases or decreases. Whereas negative correlation of CA15-3 for malignant and borderline ovarian tumor with insignificant p-value showing when cancer status change then CA15-3 increases or decreases. There is negative correlation of HE4 for FIGO stage I, II vs stage III, IV with insignificant p-value shows when cancer stage changes then HE4 level increase or decrease whereas negative correlation of Ca15-3 stage I, II and stage III, IV existed with insignificant p-value shows when cancer stage change then CA15-3 increase or decrease.

The sensitivity, specificity, PPV and NPV of HE4 for tumor type was 100%, 7.69%, 64.7% and 100% respectively. While sensitivity, specificity, PPV and NPV of HE4 for age was 57.35%, 50%, 97.5% and 3.33% respectively. While sensitivity, specificity, PPV and NPV of HE4 for FIGO stage was 100%, 5.71%, 51.5% and 100% respectively (Table 1).

ROC Curve of Tumor Markers (HE4, CA15-3, CA125

Table 1: *Sensitivity, Specificity, PPV and NPV of HE4*

Risk Factor	Category	HE4[26]		Sensitivity (%)	Specificity (%)	95% CI	PPV (%)	NPV (%)
		Normal (0-150)	Abnormal (>150)					
Tumor	Malignant	44	0	100	7.69	91.96 – 100	64.7	100
	Borderline	24	2			0.95 – 25.13		
Age	Premenopausal <50 year	39	1	57.35	50	44.77 – 69.28	97.5	3.33
	Postmenopausal ≥55 year	29	1			1.26 – 98.74		
Stage	Stage I & II	35	0	100	5.71	90 - 100	51.5	100
	Stage III & IV	33	2			0.7 – 19.16		

The sensitivity, specificity, PPV and NPV of CA15-3 for tumor type was 65%, 50%, 88.6% and 19.2% respectively. While sensitivity, specificity, PPV and NPV of CA15-3 for age was 58.33%, 50%, 87.5% and 16.7% respectively. While sensitivity, specificity, PPV and NPV of CA15-3 for FIGO stage was 50%, 50%, 85.7% and 14.2% respectively (Table 2).

Table 2: *Sensitivity, Specificity, PPV and NPV of CA15-3*

Risk Factor	Category	CA15-3[26]		Sensitivity (%)	Specificity (%)	95% CI	PPV (%)	NPV (%)
		Normal (0-38)	Abnormal (>38)					
Tumor	Malignant	39	5	65	50	51.6 – 76.87	88.6	19.2
	Borderline	21	5			18.71 – 81.29		
Age	Premenopausal <50 year	35	5	58.33	50	44.88 – 70.93	87.5	16.7
	Postmenopausal ≥50 year	25	5			18.71 – 81.29		
Stage	Stage I & II	30	5	50	50	36.81 -63.19	85.7	14.2
	Stage III & IV	30	5			18.71 - 81.29		

The sensitivity, specificity, PPV and NPV of CA125 (Follow up) for tumor type was 63.16%, 37.25%, 27.3% and 73.1% respectively. While sensitivity, specificity, PPV and NPV of CA125 (Follow up) for age was 78.95%, 50.98%, 37.5% and 86.7% respectively. While sensitivity, specificity, PPV and NPV of CA125 (Follow up) for FIGO stage was 68.42%, 56.86%, 37.1% and 82.9% respectively (Table 3).

Table 3: *Sensitivity, Specificity, PPV and NPV of CA125 Follow up*

Risk Factor	Category	CA125 Follow up[27]		Sensitivity (%)	Specificity (%)	95% CI	PPV (%)	NPV (%)
		Normal (<35)	Abnormal (>=35)					
Tumor	Malignant	12	32	63.16	37.25	38.36 – 83.71	27.3	73.1
	Borderline	7	19			24.13 – 51.92		
Age	Premenopausal <50 year	15	25	78.95	50.98	54.43 – 93.95	37.5	86.7
	Postmenopausal ≥50 year	4	26			36.6 – 65.25		
Stage	Stage I&II	13	22	68.42	56.86	43.45 - 87.42	37.1	82.9
	Stage III&IV	6	29			42.25 - 70.65		

baseline & follow up)

The detection worth of tumor markers HE4, CA15-3 and CA125 (baseline and follow up) can be narrated with ROC curve. Any tested serum marker which possess AUC >0.50% is considered to have discriminative strength for the diagnosis or prognosis of ovarian cancer.

ROC Curve of Figure 1-a displays the AUC of these tumor markers (HE4, CA15-3, CA 125 baseline & follow up) for tumor type (malignant and borderline) of ovarian cancer as follows 0.55%, 0.59%, 0.48% and 0.43% respectively.

Similarly ROC curve in figure 1-b presents AUC for these markers (HE4, CA15-3, CA125 baseline & follow up) for age groups (premenopausal and post-menopausal) and have these values of 0.41%, 0.55%, 0.30% and 0.30% respectively.

Moreover ROC curve in figure 1-c shows following values of AUC of (HE4, CA15-3, CA125 baseline & follow up)

For FIGO stages (I, II and III, IV) 0.47%, 0.66%, 0.30% and 0.33% respectively.

Thus, the goodness of testing any of these tumor markers individually or in combination with any of associated factor of ovarian cancer like (age, tumor type or FIGO stage) is evident from present study. Thus testing these biomarkers could be cost effective and non-invasive tool of assessment of disease.

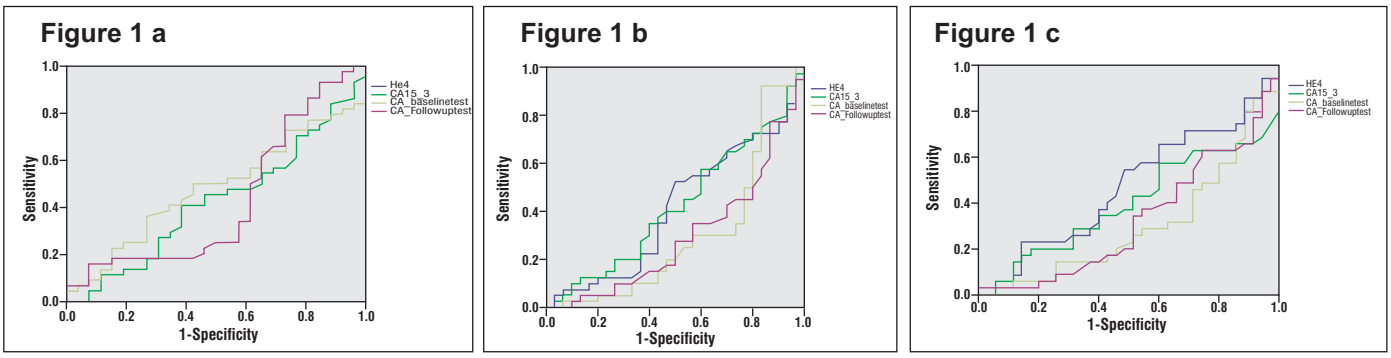

Figure 1: *ROC Curves of HE4, CA15-3 & CA125 (BASELINE AND FOLLOW UP).*
Figure 1-a: AUC for HE4, CA15-3 & CA125 (Baseline and follow up) for Tumor Type
1-b: AUC for HE4, CA15-3 & CA125 (Baseline and follow up) for Age
1-c: AUC for HE4, CA15-3 & CA125 (Baseline and follow up) for FIGO Stage
The area under curve for HE4, CA15-3, CA125 baseline & follow-up is 0.55, 0.43, 0.48 and 0.43 respectively for tumor type (malignant & borderline) (Figure-1a)
The area under curve for HE4, CA15-3, CA125 baseline & follow-up is 0.42, 0.43, 0.30 and 0.30 respectively for age (Figure-1b)
The area under curve for HE4, CA15-3, CA125 baseline & follow-up is 0.47, 0.40, 0.30 and 0.33 respectively for FIGO Stage (Figure-1c)

Discussion

The cancer associated specific biomarkers are yet under research for diagnosis and prognosis of ovarian cancer. Tumors usually shed certain proteins in blood that can be detected as powerful indicator in varied tumors. Determining HE4 and CA15-3 in ovarian cancer has proved effective in defining sensitivity and specificity worth of these markers among Asian and other populations. Literature review shows wide range of sensitivity and specificity of HE4, CA15-3 determined in different ethnicities. In this context this study explored serum levels of HE4 and CA15-3 in women of Pakistan and found important results. The relationship between diagnostic sensitivity and specificity was expressed by ROC curve and AUC, PPV, NPV of HE4, CA15-3, CA125 (baseline & follow up) alone and in combination for age (pre -menopausal (<50year), post- menopausal (≥50year), FIGO stage (early I &II, advanced III & IV) was also assessed.

This study reported mean age 45.63 years of ovarian cancer women who participated in this research. This figure is consistent with another study who showed mean age (45.58±1.27, 46).[28] But this age presentation of this study is different from another study whereby reported mean age of cancer patients was 53.8 ± 14.9 years (32-87 years).[9]

This study reported mean level of HE4=20.79 pmol/L (5.19pmol/L - 268.5pmol/L). This is in contrast to other studies who found mean HE4= 288.63±135.67 pmol/Ml[29], and another research who presented mean HE4 119 ± 123.[23] pmol/mL (4pmol/mL-567.21pmol/mL).[26] While, another study reported mean HE4=75.7 pmol/mL and 1338.0 for pmol/mL benign vs malignant groups respectively.[1] The reason for difference in finding could be unequal samples of FIGO stages.

This study determined mean CA125 baseline 1126.34 U/mL and follow up 77.0 U/mL and this is also in contrast to values given by another study whereby mentioned mean value of CA125 for benign vs malignant group is 195.517 U/mL & 1763.4 U/mL respectively.[1] Another study presented mean value of CA 125, 42.1±176.324.

Mean level of CA15-3 found in our study were, 51.50 U/mL (0- U/mL -796.2 U/mL) which is different from others results 75.83± 289.4 IU/L (0 IU/L -2412 IU/L).[27]

This research explored positive correlation of HE4 among malignant and borderline ovarian tumors r = 0.089, p=0.665 whereas negative correlation r = -0.028 p=0.875 among early (I & II) vs advanced (III & IV) FIGO stage.

Also this study discovered negative correlation r = -0.005 p= 0.980 for CA15-3 both for malignant vs borderline ovarian tumors as well as early (I, II) vs advanced (III, IV) FIGO stage with r= -0.095 p=0.588. These results are in contrast to another study whereby no correlation was discovered between CA125 and CA15-3 levels of advanced epithelial ovarian cancer patients.[30] The reason of different result is varied histological types of ovarian cancer.

This study determined sensitivity, specificity, PPV, NPV for Ca125 (follow up) 63.16%, 37.25%, 27.27%, 73.08% respectively in comparison with 83.3 % 85% 80.7 % 87.2%15 also another study presented 73.2%, 79.2%,[30] and another study showed 87.4%, 80.5%, 78.3%, 88.8% respectively.[24]

This research explored sensitivity, specificity, PPV, NPV for HE4 64.71%,100%,100%,7.69% respectively in comparison with other study results with sensitivity and specificity 82.9% & 87.5% respectively[30] and others presented 90%,95%,93.1%,92.7% respectively.[15]

Also this study determined sensitivity, specificity, PPV, NPV for CA15-3 65%,50%,88.64%,19.23% respectively in comparison with other study who showed 53.13%, 96.08%, 89.47%, 76.56% respectively.[26] Another research presented following values of sensitivity, specificity, PPV, NPV for CA15-3 88.4%, 79.7%, 78.6%, 89.5% & 88.4%, 80.1%, 78.9%, 89.5% respectively with cut off at 35 and 4024.

ROC (Receiver Operating Characteristic)-AUC (Area Under Curve) of Tumor Markers CA125, HE4 and CA15-3: This research reported AUC of CA125 (baseline and follow up) for tumor type (malignant and borderline) 0.48% 0.43%. In comparison with 0.83% (95% CI: 0.71-0.92; p<0.0001)9. Also another study presented AUC 0.914% (95% CI; 0.887-0.941)[24] whereby the study groups were women with benign/malignant and borderline adnexal masses.

This research presented AUC for HE4 =0.55% for tumor type (malignant and borderline), In comparison with 0.84% (95% CI: 0.72-0.92; p<0.0001) VS9 their research groups were ovarian cancer vs healthy controls.

Also another study presented AUC 0.834; 95% CI; 0.794-0.87424 whereby the study groups were women with benign/malignant and borderline adnexal masses. The cause of different results is unequal sample size of four FIGO stages.

This study determined AUC for CA 15-3=0.43% for tumor type (malignant and borderline). Whereas other study shown 0.84% (65.3% sensitivity 95% specificity) for CA15-3.[31]

Conclusion

The discriminative strength of HE4 as biomarker determined by ROC curve was 0.55%. Hence HE4 can be used for diagnosis or prognosis of ovarian cancer. Moreover, the sensitivity and specificity of HE4 presented in this study is statistically significant. Hence, HE4 seems a promising serum marker with diagnostic and prognostic worth in ovarian cancer.

Ethical Approval: The Ethical Review Board of University of Health Sciences approved the study vide IRB UHS/REG-17/ERC 4659.

Conflict of Interest: The authors declare no conflict of interest.

Funding Source: None

Authors' Contribution

RH: Conception and design, acquisition of data, analysis & interpretation of data, drafting of manuscript, final approval of version to be published

SK: Analysis & interpretation of data, drafting of manuscript

HUA: Acquisition of data

References

1. Hariyano, Winarto, Bismarck JL, Laila N. Modification of cut off values for HE4,CA125, the risk of malignancy index and the risk of malignancy algorithm for ovarian cancer detection in Jakarta, Indonesia. Asian Pac J Cancer Prev. 2014; 15(5):1949-1953.
2. Kehoe S. FIGO staging in ovarian carcinoma and histological subtypes. J Gynecol Oncol. 2020; 31(4): e70.
3. Liu J, Wang HL, Ma FM, Guo HP, Fang NN, Wang SS, et al. Systematic module approach identifies altered genes and pathways in four types of ovarian cancer. Mol Med Rep. 2017; 16(6): 7907-7914.
4. Tang Q, Atiq W, Mahnoor S, Abdel-Maksoud MA, Aufy M, Yaz H, et al. Comprehensively analyzing the genetic alterations, and identifying key genes in ovarian cancer. Oncol Res. 2023; 31(2): 141-156.
5. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and Ovarian Cancer: A Comprehensive Review. Cancers. 2020; 12(12): 3730.
6. Yuan Q, Song J, Yang W, Wang H, Huo Q, Yang J, et al. The effect of CA125 on metastasis of ovarian cancer: old marker new function. Oncotarget. 2017; 8(30): 50015-50022.
7. Chen Z, Liang Q, Zeng H, Zhao Q, Guo Z, Zhong R, et al. Exosomal CA125 as A Promising Biomarker for Ovarian Cancer Diagnosis. J Cancer. 2020; 11(21): 6445-6453.
8. Kang KN, Koh EY, Jang JY, Kim CW. Multiple biomarkers are more accurate than a combination of carbohydrate antigen 125 and human epididymis protein 4 for ovarian cancer screening. Obstet Gynecol Sci. 2022; 65(4): 346-354.
9. Yılmaz EP, Kumtepe Y. Endometrial and Ovarian Cancer with MR Imaging Importance of Serum HE4 and CA 125 Levels in the Extent of Disease at Evaluation. Eurasian J Med. 2016; 48(3): 192-198.
10. Abu Hassaan SO. Monitoring ovarian cancer patients during chemotherapy and follow-up with the serum tumor marker CA125. Dan Med J. 2018; 65(4): B5463.
11. Kang KN, Koh EY, Jang JY, Kim CW. Multiple biomarkers are more accurate than a combination of carbohydrate antigen 125 and human epididymis protein 4 for ovarian cancer screening. Obstet Gynecol Sci, 2022; 65(4): 346–354.
12. Anastasi E, Farina A, Granato T, Colaiacovo F, Pucci B, Tartaglione S, et al. Recent Insight about HE4 Role in Ovarian Cancer Oncogenesis. Int J Mol Sci. 2023; 24(13): 10479.
13. Braicu EI, Krause CL, Torsten U, Mecke H, Richter R, Hellmeyer L, et al. HE4 as a serum biomarker for the diagnosis of pelvic masses: a prospective, multicenter study in 965 patients. BMC Cancer. 2022; 22(1): 831.
14. Suri A, Perumal V, Ammalli P, Suryan V, Bansal SK. Diagnostic measures comparison for ovarian malignancy risk in Epithelial ovarian cancer patients: a meta-analysis. Sci Rep. 2021; 11(1): 17308.
15. Yang S, Tang J, Rong Y, Wang M, Long J, Chen C, et al. Performance of the IOTA ADNEX model combined with HE4 for identifying early-stage ovarian cancer. Front Oncol. 2022; 12: 949766.

16. AlSomairi A, Himayda S, Altelmesani A, Lee YJ, Lee JY. Prognostic value of HE4 in advanced-stage, high-grade serous ovarian cancer: Analysis of HE4 kinetics during NACT, predicting surgical outcome and recurrence in comparison to CA125. Gynecol Oncol, 2024; 181: 155-161.
17. Barr CE, Funston G, Jeevan D, Sundar S, Mounce LTA, Crosbie EJ. The Performance of HE4 Alone and in Combination with CA125 for the Detection of Ovarian Cancer in an Enriched Primary Care Population. Cancers. 2022; 14(9): 2124.
18. Barani M, Bilal M, Sabir F, Rahdar A, Kyzas GZ. Nanotechnology in ovarian cancer: Diagnosis and treatment. Life Sci. 2021; 266(1): 18914.
19. Matsas A, Stefanoudakis D, Troupis T, Kontzoglou K, Eleftheriades M, Christopoulos P, et al. Tumor Markers and Their Diagnostic Significance in Ovarian Cancer. Life (Basel). 2023;13(8): 1689.
20. Li M, Men X, Zhang X. Diagnostic value of carbohydrate antigen 72-4 combined with carbohydrate antigen 15.3 in ovarian cancer, cervical cancer and endometrial cancer. J BUON. 2020; 25(4): 1918-1927.
21. Seale KN, Tkaczuk KHR. Circulating Biomarkers in Breast Cancer. Clin Breast Cancer. 2022; 22(3): e319-e331.
22. Bel'skaya LV, Sarf EA, Loginova AI, Vyushkov DM, Choi ED. Potential Diagnostic Value of Salivary Tumor Markers in Breast, Lung and Ovarian Cancer: A Preliminary Study. Curr Issues Mol Biol. 2023; 45(6): 5084-5098.
23. Chudecka-Głaz A, Strojna A, Michalczyk K, Wieder-Huszla S, Safranow K, Skwirczyńska E, et al. Evaluation of He4 Use in the Diagnosis of Ovarian Cancer: First and Second Recurrence, and an Analysis of HE4 Concentration during Second- and Third-Line Chemotherapy. Diagnostics (Basel). 2023; 13(3): 452.
24. Sagi-Dain L, Lavie O, Auslander R, Sagi S. Clinical Use and Optimal Cutoff Value of Ca15-3 in Evaluation of Adnexal Mass: Retrospective Cohort Study and Review of the Literature. Am J Clin Oncol. 2018; 41(9): 838-844.
25. WMA, World Medical Association (2020) WMA declaration of Helsinki-ethical principles for medical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:64th WMA General Assembly, Fortaleza, Brazil, October 2013. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects.
26. Nam JE, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, et al. MicroRNA Expression Profiles in Serous Ovarian Carcinoma. Clin Cancer Res. 2008; 14(9): 2690-2695.
27. Terzic M, Dotlic J, Likic I, Nikolic B, Brndusic N, Pilic I, et al. Diagnostic value of serum tumor markers for andexal masses. Eur J Med. 2014; 9(3): 417-423.
28. Akhtar N, Rajan S, Kumar V, Sharma S, Qayoom S, Mukjarjee S, et al. Serum HE4 Detects Ovarian Cancer Recurrence Earlier Than CA 125: Indian Study. J Clin Diag Res. 2018; 12(5): XC01-XC03.
29. Xi QP, Pu DH, Lu WN. Research on application value of combined detection of serum CA125, HE4 and TK1 in the diagnosis of ovarian cancer. Eur Rev Med Pharmacol Sci. 2017; 21(20): 4536-41.
30. Gemer O, Oustinov N, Gdalevich M, Dubnik S, Levy R, Yachnin A, et al. Pretreatment CA 15-3 levels do not predict disease-free survival in patients with advanced epithelial ovarian cancer. Tumori. 2013; 99(2): 257-60.
31. Dolscheid-Pommerich RC, Keyver-Paik M, Hecking T, Kuhn W, Hartmann G, Stoffel-Wagner B, et al. Clinical performance of LOCI™-based tumor marker assays for tumor markers CA 15-3, CA 125, CEA, CA 19-9 and AFP in gynecological cancers. Tumour Biol. 2017; 39 (10): 1010428317730246.