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Serum HE4 and CA-125 in Relation to Oxidative Stress and Antioxidant Status in Women with Benign and Malignant Ovarian Tumors

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Abstract

Background: Clinical examination alone is still challenging to differentiate ovarian cancer from benign ovarian illness. In this study, we assessed the combined use of tumor markers HE4 and CA-125 with oxidative-stress indicators in an effort to obtain a wider biochemical profile of ovarian illness.
Materials and Methods: The case-control study consisted of 105 women, 35 women in each of three groups: healthy controls, benign ovarian tumor patients, and women with stage III, grade 3 ovarian cancer. Serum HE4 and CA-125 were tested by ELISA kits. Total oxidant status (TOS), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured by colorimetric method and oxidative stress index (OSI) was computed from TOS and TAC. We assessed group comparisons, receiver operating characteristic analysis, correlations and odds ratios.
Results: In controls, the level of HE4 was 27.42 ± 5.21 pmol/L, in benign illness 58.64 ± 8.63 pmol/L and in malignancy 183.32 ± 41.93 pmol/L. CA-125 rose from 11.95 ± 1.79 to 80.28 ± 17.54 to 548.83 ± 93.55 U/mL. TOS, MDA and OSI showed a gradual increase but TAC showed a substantial decrease among groups ($P<0.001$). CA-125 and HE4 had excellent discriminating between malignant and benign illness with AUCs of 1.000 and 0.998, respectively; OSI also performed well (AUC=0.941). Tumor markers were positively connected with oxidant indicators and negatively correlated with TAC.
Discussion: The results show a gradual transition from the healthy status to the benign disease and advanced malignancy with increasing values of tumor markers and increased oxidative imbalance. We suggest that integration of tumor related and redox indicators may give supplementary information on ovarian disease severity. These results favor integrated assessment over dependence on a single circulating marker.

Keywords: Ovarian cancer; HE4; CA-125; Oxidative stress; Oxidative stress index.

1. Introduction

Ovarian cancer is one of the most difficult to be diagnosed at an early clinically favorable stage gynecologic cancers. Early sickness may be asymptomatic, or non-specific [1]. Global estimates of cancer continue to show a large burden of ovarian malignancy. Ovarian cancer has a dismal mortality profile largely because of the significant percentage of individuals presenting with advanced disease [2]. The molecular intricacy of ovarian cancers adds to the problem in that benign ovarian lesions, borderline conditions and malignant tumors may not be distinguishable by clinical and radiographic means [3]. This is why laboratory biomarkers are sometimes used in conjunction with imaging and specialized evaluation rather than as a stand-alone diagnostic test. The most well-known circulating biomarker still in regular usage is cancer antigen 125 (CA-125) [4], Human epididymis protein 4 (HE4) has emerged as an important supplementary marker with generally higher specificity for epithelial ovarian carcinoma [5].

CA-125 is a high molecular weight glycoprotein that can be highly increased in epithelial ovarian cancer, especially in patients with more widespread illness [6]. The major limitation of it is that increased levels are not specific for cancer and can be found in a number of benign gynecology and inflammatory disorders. [7]. HE4, encoded by WFDC2, is expressed in several subtypes of epithelial ovarian cancer and has been introduced to improve the

discrimination between benign and malignant adnexal masses [8]. Recent reviews and meta-analyses have established that HE4 and CA-125 provide clinically useful but different information. CA-125 is frequently more sensitive, although HE4 is usually more specific. Thus, their combined assessment remains physiologically and therapeutically desirable, especially for the purpose of distinguishing women with benign ovarian illness from women with malignant tumors [9, 10].

The evaluation of ovarian cancer is not only the question of tumor-associated proteins. Malignant transformation includes substantial changes in cellular metabolism, mitochondrial function, inflammatory signaling and redox regulation [11]. Reactive oxygen species are formed during normal metabolism but oxidative stress occurs when excess synthesis of reactive oxygen species occurs or when antioxidant defense is inadequate. Oxidative stress plays a dual role in cancer [12]. Sustained and moderate reactive oxygen species can promote proliferation, genomic instability, angiogenesis, invasion, and treatment adaptability. Excessive oxidative injury can be cytotoxic [13]. Ovarian cancer cells thus operate in a finely regulated redox milieu in which oxidant generation and antioxidant buffering are continuously tuned to enhance tumor survival [14].

Systemic oxidative state can be studied by complementary assays and not by a single molecule. Total oxidant status (TOS) indicates the total oxidant burden of the serum, while total antioxidant capacity (TAC) represents the entire capacity of circulating antioxidants to counteract oxidation. The oxidative stress index (OSI) calculated by the ratio of TOS to TAC, may be used as an integrated expression of the balance between the two systems [15]. Malondialdehyde (MDA) is a persistent result of lipid peroxidation and is commonly employed as a marker for oxidative damage to membrane lipids. Increased lipid peroxidation with decreased antioxidant defenses has been observed in earlier clinical research in ovarian cancer and more current work focusing on redox continues to support a trend toward pro-oxidant conditions in malignant ovarian illness [16].

Although HE4 and CA-125 have been extensively studied as diagnostic biomarkers and the independent role of oxidative stress in ovarian carcinogenesis is well established, there are few clinical studies evaluating these two domains of biomarkers together in healthy women, women with benign ovarian tumors and women with clearly advanced malignant disease within the same analytical framework. This combined method may be very useful since it considers the tumor-associated signal indicated by HE4 and CA-125 in combination with the systemic biochemical environment represented by TOS, TAC, MDA and OSI. The simultaneous study of these variables may explain if successive changes in tumor indicators are associated with a corresponding shift toward oxidant dominance and may also reveal whether redox markers provide descriptive or discriminating information beyond traditional tumor markers.

Thus, the present study was planned to examine serum HE4, CA-125, TOS, TAC, MDA and computed OSI in apparently healthy women, women with benign ovarian tumors, and women with malignant ovarian cancer categorized as stage III and grade 3. The study also attempted to analyze the diagnostic discrimination of the investigated markers, to define their inter-relationships and to determine the intensity of correlation of selected cut-off values with benign and malignant ovarian disease. The aim of the study was to combine known ovarian tumor indicators with markers of oxidative and antioxidant status to create a wider biochemical picture of the transition from a healthy state to benign ovarian disease and further to more serious malignancy.

2. Materials and Methods

2.1 Study Design and Participants

This case-control research comprised 105 women who were separated into three equal groups (n = 35 each group). Control group consisted of women who seemed healthy and were not known to have any ovarian disease. The benign group was comprised of women diagnosed with benign ovarian tumors, whereas the malignant group was comprised of women diagnosed with malignant ovarian cancer. All women in the malignant group were staged as stage III, grade 3 both clinically and pathologically. The recruitment and blood collection were obtained from private medical laboratories after clinical assessment by a specialist physician in gynecology/oncology. The diagnostic classification of the patient groups was based on the specialist's evaluation and the clinical and pathological records available. The patients and participants in the study gave their consent orally.

2.2 Inclusion and Exclusion Criteria

Women were eligible for the benign group when a specialist physician had detected a benign ovarian tumor. Women were eligible for the malignant group when ovarian cancer was proven and the reported disease was stage III, grade 3. Women included in the controls were apparently healthy and with no known ovarian malignancy. Women with other significant chronic conditions that might independently alter systemic oxidative state were excluded. The major exclusion criteria included hypertension, diabetes mellitus, renal failure, other major chronic conditions and active smoking. These parameters were utilized to minimize major non-ovarian impacts on oxidative stress and antioxidant measures.

2.3 Blood Collection and Serum Preparation

Venous blood (5 mL) was obtained aseptically from each subject. The blood was allowed to coagulate and then centrifuged at 5000 rpm for 5 min to separate the serum. The clear serum fraction was placed into clean labeled tubes and stored at −4 °C for laboratory examination. Avoided repeated unneeded freeze-thaw cycles. The stored serum samples were gently mixed and equilibrated to suitable working temperature prior to analysis.

2.4 Measurement of Serum HE4 by ELISA

Serum HE4 was determined using a commercial Human Epididymis Protein 4 (HE4) ELISA kit (Catalogue No. SL1946Hu, SunLong Biotech Co., Ltd., Hangzhou, China). The assay was run as a sandwich ELISA according to the manufacturer's instructions. The kit has a measurement range of 2-40 pmol/L and a sensitivity of 0.5 pmol/L. Reagents and serum samples were equilibrated to appropriate working temperature before to use. Prepared standards and serum samples were then added to the wells of the antibody coated microplate and incubated for binding of HE4. The unbound material was rinsed off and the enzyme conjugated detection reagent was added and the plate was incubated again. The second wash step was followed by the addition of chromogenic substrate and development of color. After addition of stop solution, the reaction was stopped and the optical density (OD) was measured at 450 nm. The serum HE4 concentrations were determined from the standard calibration curve and expressed in pmol/L. Samples exceeding the analytical range were diluted proportionately and the final concentration corrected for dilution.

2.5 Measurement of Serum CA-125 by ELISA

Serum CA-125 was measured by Human Carbohydrate Antigen 125 (CA-125) ELISA Kit (SunLong Biotech Co., Ltd, Hangzhou, China; Catalog No. SL2229Hu). The assay was performed using the sandwich ELISA technique according to manufacturer's instructions. The analytical range of the kit was 0.3–20 U/mL and the sensitivity was 0.05 U/mL. Standards and serum samples were added to the antibody-coated microplate wells and incubated. The wells were then washed to eliminate unbound components. Then the enzyme conjugated detection reagent was added and incubated on the plate again. A chromogenic substrate was applied to develop the color, and then a stop solution was added after washing. Optical density was read at 450 nm and serum CA-125 concentrations were determined using the matching standard curve and expressed in U/mL. Samples having concentrations beyond the analytical range were diluted as needed and the final values were corrected for the dilution factor.

2.6 Determination of Total Oxidant Status (TOS)

Serum total oxidant status was measured according to the colorimetric method in the laboratory protocol provided [17]. The process is based on the oxidation of ferrous to ferric ions by oxidants present in serum under acid conditions. The ferric ions subsequently react with xylenol orange to generate a colored complex, the intensity of which is proportional to the total oxidant number of the sample. Serum was mixed with reagent A and the first absorbance was read. Reagent B was added and the reaction mixture was incubated for 4 minutes before the final absorbance was measured spectrophotometrically. A calibration curve was constructed using hydrogen peroxide standards and the TOS was represented as μmol of H2O2 equivalent/L (μmol H2O2 Eq/L).

2.7 Determination of Total Antioxidant Capacity (TAC)

Serum total antioxidant capacity was measured using the colorimetric method described in the supplied laboratory procedure [18]. The assay measures the total capacity of the serum antioxidants to prevent an oxidation reaction.

Serum and Reagent A were mixed and the first absorbance was recorded. Reagent B containing the oxidizing component was then added and the mixture was incubated for 4 min to allow the final absorbance to be recorded. Standards of ascorbic acid were used to produce a standard curve. TAC findings were reported in mmol/L.

2.8 Determination of Malondialdehyde (MDA)

Serum MDA was assessed using the thiobarbituric acid reaction as a marker for lipid peroxidation. Briefly, 500 µL of serum was combined with 500 µL of 35% trichloroacetic acid and 500 µL of 50 mmol/L TrisHCl buffer (pH 7.4) and incubated at room temperature for 15 min. Then 1 ml of thiobarbituric acid reagent was added and the tubes were heated in a boiling water bath for 45 min. The samples were then cooled and 1 mL of 70% trichloroacetic acid was added and the tubes centrifuged at about 950 ×g for 10 minutes. Absorbance of supernatant was read at 532 nm. MDA concentration was determined using a molar extinction coefficient of 1.56 × 10 5 M-1cm-1 and expressed as nmol/Ml [19].

2.9 Calculation of Oxidative Stress Index (OSI)

OSI was calculated to express the balance between total oxidant burden and total antioxidant defense. Because TOS was expressed in µmol/L and TAC in mmol/L, TAC was first converted to µmol/L. OSI was then calculated as: OSI = [TOS (µmol/L) / TAC (µmol/L)] × 100. The index was reported in arbitrary units.
A higher OSI indicates a greater shift toward oxidant predominance.

2.10 Statistical Analysis

Continuous variables are presented as mean ± standard deviation. Welch's one-way analysis of variance was used to analyze differences across the control, benign and malignant groups because the dispersion of multiple biomarkers changed greatly among groups. Discriminatory performance was summarized using receiver operating characteristic (ROC) analysis including the area under the curve (AUC), appropriate cut-off, sensitivity and specificity. Correlation matrices were shown for control + benign participants and for control + malignant participants. The relationship between specified biomarker cut-offs and group status was presented as odds ratios (ORs) with 95% confidence intervals. Statistical significance was defined as a P value < 0.05.

3. Results

Table 1 summarizes the mean concentrations of the investigated tumor markers and oxidative-stress parameters in the control, benign, and malignant ovarian groups and presents the overall probability value for the three-group comparison.

Table 1: Comparison of study parameters among control, benign, and malignant ovarian groups.

Parameter	Control (n=35) Mean ± SD	Benign (n=35) Mean ± SD	Malignant (n=35) Mean ± SD	P value
HE4 (pmol/L)	27.42 ± 5.21	58.64 ± 8.63	183.32 ± 41.93	<0.001
CA-125 (U/mL)	11.95 ± 1.79	80.28 ± 17.54	548.83 ± 93.55	<0.001
TOS (µmol/L)	7.91 ± 2.17	11.42 ± 3.23	16.25 ± 2.71	<0.001
MDA (nmol/mL)	2.90 ± 0.80	4.91 ± 1.10	5.80 ± 1.20	<0.001
TAC (mmol/L)	1.62 ± 0.24	1.05 ± 0.20	0.83 ± 0.11	<0.001
OSI (AU)	0.49 ± 0.15	1.09 ± 0.37	1.96 ± 0.42	<0.001

HE4 increased from 27.42 ± 5.21 pmol/L in controls to 58.64 ± 8.63 pmol/L in the benign group and 183.32 ± 41.93 pmol/L in the malignant group. CA-125 showed a similar stepwise increase, with mean values of 11.95 ± 1.79, 80.28 ± 17.54, and 548.83 ± 93.55 U/mL, respectively. TOS and MDA also increased progressively across the three groups, whereas TAC decreased from 1.62 ± 0.24 mmol/L in controls to 1.05 ± 0.20 mmol/L in benign disease and 0.83 ± 0.11 mmol/L in malignant disease. OSI increased from 0.49 ± 0.15 to 1.09 ± 0.37 and 1.96 ± 0.42 AU. The overall group difference was P<0.001 for all six parameters.

Table 2 shows the ROC features of HE4, CA-125, TOS, MDA, TAC and OSI for discrimination of benign ovarian group from healthy controls.

Table 2: ROC analysis for benign ovarian disease versus controls.

Marker	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)	P value
HE4 (pmol/L)	0.999	0.991–1.000	≥39.89	98.5	99.2	<0.001
CA-125 (U/mL)	1.000	1.000–1.000	≥19.26	100.0	100.0	<0.001
TOS (µmol/L)	0.816	0.715–0.917	≥10.05	66.4	83.8	<0.001
MDA (nmol/mL)	0.930	0.867–0.993	≥3.88	82.5	89.0	<0.001
TAC (mmol/L)	0.966	0.922–1.000	≤1.32	91.5	89.1	<0.001
OSI (AU)	0.933	0.871–0.995	≥0.74	82.8	94.9	<0.001

CA-125 produced an AUC of 1.000 at a cut-off of ≥19.26 U/mL, with sensitivity and specificity of 100.0% each. HE4 produced an AUC of 0.999 at ≥39.89 pmol/L, with sensitivity of 98.5% and specificity of 99.2%. Among the oxidative-stress variables, TAC, OSI, and MDA produced AUC values of 0.966, 0.933, and 0.930, respectively, whereas TOS produced an AUC of 0.816.

Table 3 shows the ROC characteristics of the same marker panel for discrimination of malignant ovarian cancer from benign ovarian disease.

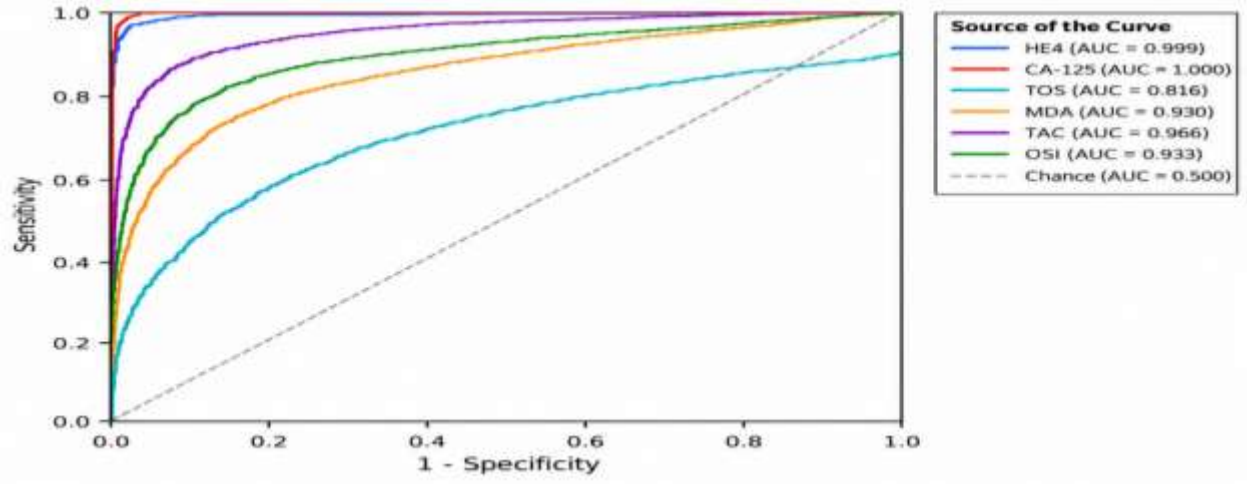


Figure 1: *Roc analysis for benign patients*

Table 3: ROC analysis for malignant versus benign ovarian disease.

Marker	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)	P value
HE4 (pmol/L)	0.998	0.987–1.000	≥84.17	99.1	99.8	<0.001
CA-125 (U/mL)	1.000	1.000–1.000	≥159.94	100.0	100.0	<0.001
TOS (µmol/L)	0.874	0.790–0.958	≥13.73	82.4	76.3	<0.001
MDA (nmol/mL)	0.708	0.586–0.830	≥5.46	61.1	69.2	<0.001
TAC (mmol/L)	0.832	0.735–0.929	≤0.96	88.1	67.4	<0.001
OSI (AU)	0.941	0.883–0.999	≥1.52	85.4	87.7	<0.001

The AUC for CA-125 for malignant vs benign disease was 1.000 at a cut-off of ≥159.94 U/mL and for HE4 it was 0.998 at a cut-off of ≥84.17 pmol/L. The AUC of OSI was 0.941 followed by TOS (0.874) and TAC (0.832). The lowest AUC in this comparison was for MDA (0.708).

Table 4 displays the correlation matrix for the benign group, which indicates the direction and amount of the connections among the six measured or calculated variables.

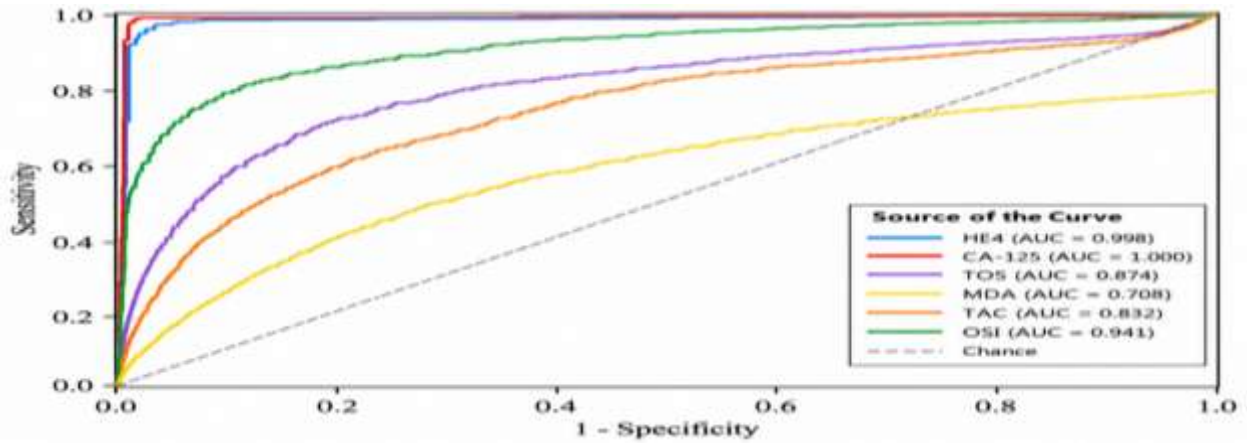


Figure 2: *Roc analysis for malignant patients*

Table 4: Correlation matrix for benign group (n=35).

Variable	HE4	CA-125	TOS	MDA	TAC	OSI
HE4	1.000 (—)	0.858 (<0.001)	0.495 (0.003)	0.663 (<0.001)	-0.725 (<0.001)	0.667 (<0.001)
CA-125	0.858 (<0.001)	1.000 (—)	0.511 (0.002)	0.685 (<0.001)	-0.748 (<0.001)	0.688 (<0.001)
TOS	0.495 (0.003)	0.511 (0.002)	1.000 (—)	0.395 (0.019)	-0.432 (0.010)	0.869 (<0.001)
MDA	0.663 (<0.001)	0.685 (<0.001)	0.395 (0.019)	1.000 (—)	-0.578 (<0.001)	0.532 (0.001)
TAC	-0.725 (<0.001)	-0.748 (<0.001)	-0.432 (0.010)	-0.578 (<0.001)	1.000 (—)	-0.776 (<0.001)
OSI	0.667 (<0.001)	0.688 (<0.001)	0.869 (<0.001)	0.532 (0.001)	-0.776 (<0.001)	1.000 (—)

In -benign, HE4 correlated positively with CA-125 (r=0.858), TOS (r=0.495), MDA (r=0.663), and OSI (r=0.667), and negatively with TAC (r=-0.725). CA-125 also correlated positively with TOS (r=0.511), MDA (r=0.685), and OSI (r=0.688), and negatively with TAC (r=-0.748). TOS showed its strongest positive relationship with OSI (r=0.869), while TAC showed a negative relationship with OSI (r=-0.776).

Table 5 presents the corresponding correlation matrix for malignant groups.

Table 5: Correlation matrix for malignant group (n=35).

Variable	HE4	CA-125	TOS	MDA	TAC	OSI
HE4	1.000 (—)	0.909 (<0.001)	0.809 (<0.001)	0.769 (<0.001)	-0.848 (<0.001)	0.862 (<0.001)
CA-125	0.909 (<0.001)	1.000 (—)	0.841 (<0.001)	0.799 (<0.001)	-0.881 (<0.001)	0.896 (<0.001)
TOS	0.809 (<0.001)	0.841 (<0.001)	1.000 (—)	0.711 (<0.001)	-0.784 (<0.001)	0.946 (<0.001)
MDA	0.769 (<0.001)	0.799 (<0.001)	0.711 (<0.001)	1.000 (—)	-0.745 (<0.001)	0.757 (<0.001)
TAC	-0.848 (<0.001)	-0.881 (<0.001)	-0.784 (<0.001)	-0.745 (<0.001)	1.000 (—)	-0.900 (<0.001)
OSI	0.862 (<0.001)	0.896 (<0.001)	0.946 (<0.001)	0.757 (<0.001)	-0.900 (<0.001)	1.000 (—)

In the control-plus-malignant comparison, HE4 correlated positively with CA-125 (r=0.909), TOS (r=0.809), MDA (r=0.769), and OSI (r=0.862), and negatively with TAC (r=-0.848). CA-125 showed positive correlations with TOS (r=0.841), MDA (r=0.799), and OSI (r=0.896), together with an inverse correlation with TAC (r=-0.881). The strongest positive coefficient in the matrix was observed between TOS and OSI (r=0.946), whereas the strongest inverse coefficient was between TAC and OSI (r=-0.900).

Table 6 shows the odds ratios associated with the selected biomarker cut-offs for benign ovarian disease relative to the control group.

Table 6: Odds ratios for benign ovarian disease versus controls.

Criterion	OR	95% CI	P value
HE4 ≥39.89 pmol/L	1521.97	77.97–29709.63	<0.001
CA-125 ≥19.26 U/mL	4944.70	97.22–251496.31	<0.001
TOS ≥10.05 µmol/L	10.24	3.27–32.05	<0.001
MDA ≥3.88 nmol/mL	38.23	9.69–150.85	<0.001
TAC ≤1.32 mmol/L	87.92	17.86–432.81	<0.001
OSI ≥0.74 AU	89.33	15.64–510.27	<0.001

For benign disease versus controls, the largest odds ratio was observed for CA-125 ≥19.26 U/mL (OR=4944.70), followed by HE4 ≥39.89 pmol/L (OR=1521.97). The ORs for OSI ≥0.74 AU and TAC ≤1.32 mmol/L were 89.33 and 87.92, respectively. MDA ≥3.88 nmol/mL had an OR of 38.23, while TOS ≥10.05 µmol/L had an OR of 10.24.

Figure 3: odds ratio for benign disease versus control group.

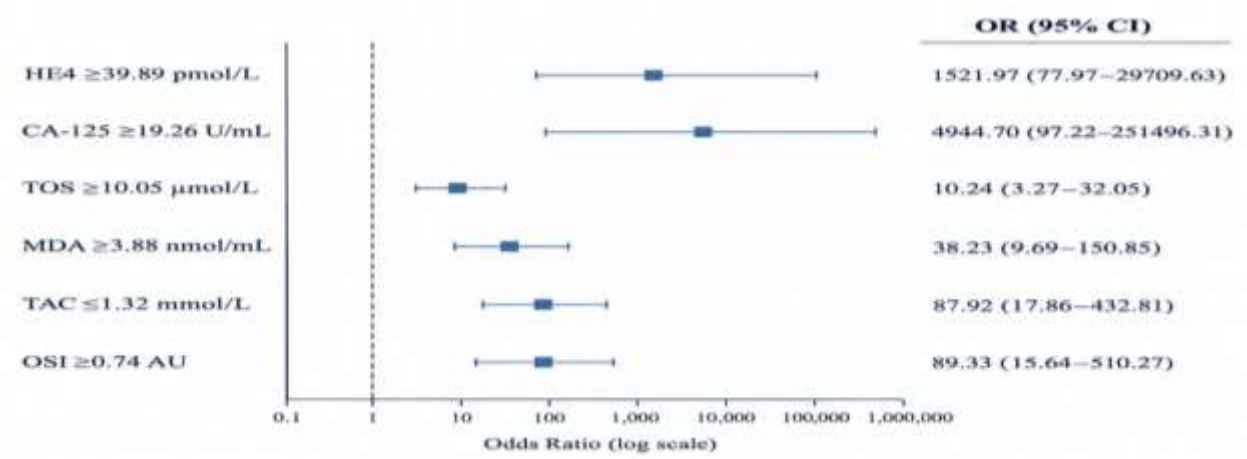


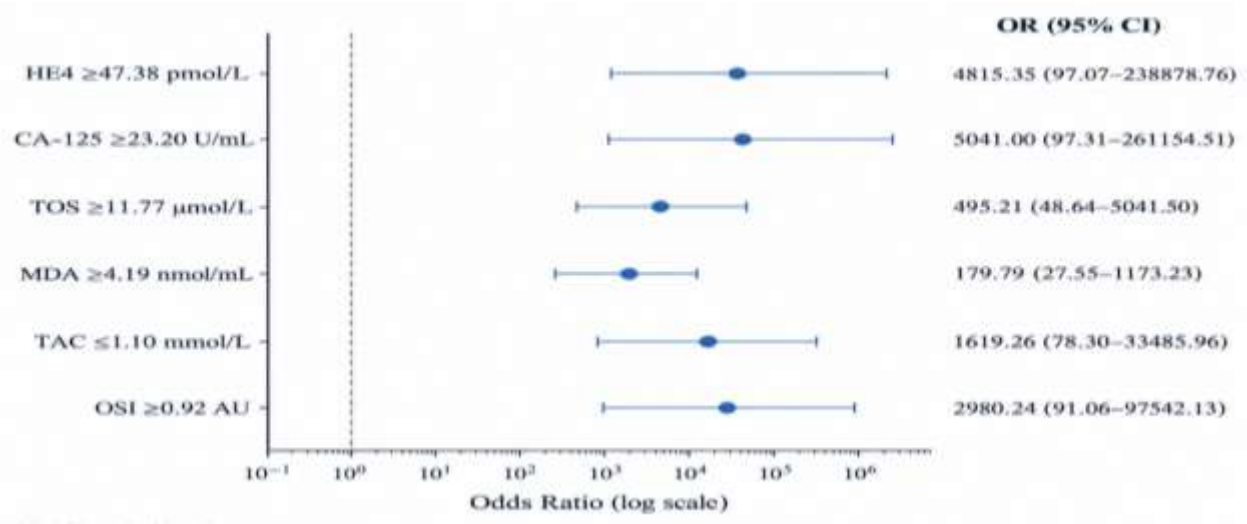
Table 7 presents the odds ratios for malignant ovarian cancer relative to controls using the selected cut-off values.

Table 7: Odds ratios for malignant ovarian cancer versus controls.

Criterion	OR	95% CI	P value
HE4 ≥47.38 pmol/L	4815.35	97.07–238878.76	<0.001
CA-125 ≥23.20 U/mL	5041.00	97.31–261154.51	<0.001
TOS ≥11.77 µmol/L	495.21	48.64–5041.50	<0.001
MDA ≥4.19 nmol/mL	179.79	27.55–1173.23	<0.001
TAC ≤1.10 mmol/L	1619.26	78.30–33485.96	<0.001
OSI ≥0.92 AU	2980.24	91.06–97542.13	<0.001

For malignant ovarian cancer versus controls, CA-125 ≥23.20 U/mL produced the highest OR (5041.00), followed by HE4 ≥47.38 pmol/L (OR=4815.35). OSI ≥0.92 AU produced an OR of 2980.24, TAC ≤1.10 mmol/L an OR of 1619.26, TOS ≥11.77 µmol/L an OR of 495.21, and MDA ≥4.19 nmol/mL an OR of 179.79.

Figure 4: *Odds ratio for malignant disease versus control group.*



4. Discussion

The present investigation indicated a distinct biochemical gradient between healthy women, women with benign ovarian tumors and women with stage III, grade 3 ovarian cancer. There was a progressive increase in HE4 and CA-125 from the control to benign disease and the highest amounts were observed in the malignant group. Meanwhile the oxidative pattern was altered to a more oxidant state: TOS and MDA increased, TAC decreased and OSI increased significantly. This general pattern is biologically plausible as malignant ovarian illness is characterized not only by increased production of tumor-associated proteins but also by abnormal energy metabolism, inflammation, mitochondrial dysfunction and redox adaption. Recent studies emphasize that ovarian cancer cells utilize reactive oxygen species as signaling molecules and reprogram antioxidant defenses to escape deadly oxidative damage [20, 21]. The global clinical importance of identifying such changes is reinforced by the continuing burden of ovarian cancer and the persistent problem of advanced-stage presentation [22].

The substantial rise of CA-125 in the malignant group is in keeping with its well documented clinical association with epithelial ovarian cancer. In the present findings CA-125 rose from a low mean concentration in controls to an intermediate concentration in benign ovarian illness and a substantially higher level in malignant patients. This pattern in steps is expected in that CA-125 may increase in many benign gynecologic diseases but very high levels are more often found when the burden of epithelial ovarian cancer is large. CA-125 remains the most extensively used serum marker in ovarian cancer, but its specificity is hampered when benign adnexal illness is the competing diagnosis. 1-3 Recent diagnostic reviews have continued to identify CA-125 as the most widely used serum marker in ovarian cancer [23, 24]. The 2025 comparative meta-analysis by Singh et al. similarly found that CA-125 retained strong sensitivity but was less specific than HE4 and multimarker indice [10]. Therefore, the strong separation observed in this cohort is compatible with the advanced stage and high grade of the malignant group, although such separation would be expected to be less pronounced in populations containing a larger proportion of early-stage cancers.

HE4 was likewise significantly elevated in all groups of the research and demonstrated excellent discrimination. This conclusion is in line with the current literature that considers HE4 as an essential supplementary marker to CA-125. Suri et al. in their meta-analysis of epithelial ovarian cancer observed greater pooled specificity for HE4 than for CA-125, while Nalini et al. reported serum HE4 to have a pooled specificity of around 0.92 in a large updated diagnostic dataset [25]. Ghose et al. likewise emphasized that HE4 and CA-125 remain central components of contemporary serum-biomarker strategies, even as newer molecular markers are investigated [23]. The substantial positive connection between HE4 and CA-125 in the correlation analyzes of the present study is therefore justified, as both markers increase with increasing malignant tumor burden, although they represent different biological molecules and are affected by different non-malignant variables. In addition, HE4 has been proven in surveillance studies to be capable of monitoring disease state in epithelial ovarian cancer generally similar to CA-125 [26].

ROC analysis demonstrated that HE4 and CA-125 showed almost perfect discriminating in the present dataset. The AUC values for benign disease against controls were 0.999 for HE4 and 1.000 for CA-125, and similarly high values were reported for malignant versus benign disease. These estimations are greater than the pooled AUCs generally reported in external clinical research. For example, Suri et al. reported AUC values of approximately 0.91 for HE4 and 0.86 for CA-125, but the 2025 comparative meta-analysis revealed AUC values of approximately 0.86 for HE4 and 0.81 for CA-125 [24]. Such a difference is not surprising as the current malignant group contained only stage III, grade 3 disease, and therefore was more biologically different from healthy and benign groups than mixed stage diagnostic groups. A recent multimodal investigation of adnexal masses also provides evidence that the addition of supplementary serum information to CA-125 and HE4 can improve discriminating between benign and malignant lesions [27].

Oxidative stress results were in agreement with increases in HE4 and CA-125. TOS increased among controls, benign illness and malignancy whereas TAC decreased. The level of OSI was elevated around four-fold from control to malignant illness. This pattern is consistent with a systemic transition to oxidant dominance. In a recent summary of findings, Long et al. reported that ovarian cancer cells exist in a reprogrammed redox state, in which reactive oxygen species (ROS) drive proliferation, invasion, metabolic plasticity and resistance pathways, while antioxidant systems are reprogrammed to maintain viability [20]. Lee et al. further emphasized the context-dependent roles for reactive oxygen species in ovarian cancer, such as promoting metastatic and inflammatory signals, participating in treatment stress, or causing cell death when redox buffering capacity is exceeded [28]. These data give a mechanistic basis for the concomitant increase in TOS and decrease in TAC as seen in the present investigation.

This decline in TAC is particularly relevant since it indicates that the increase in oxidant burden is not being completely balanced by the circulating antioxidant capacity. TAC is the result of the action of a large number of low molecular weight antioxidants and proteins, so it can capture larger systemic alterations than a single antioxidant enzyme. The inverse correlations of TAC with HE4, CA-125, TOS, MDA and OSI are compatible with a progressive depletion of the antioxidant reserve when the biochemical characteristics of ovarian illness become apparent. A recent clinical study by Karabulut et al., which used thiol-disulfide homeostasis instead of TAC, reached a similar conclusion in conceptual terms: women with ovarian cancer had lower native and total thiol-related antioxidant measures and a shift toward oxidized disulfide forms compared with benign neoplasia and healthy women [29]. Although the analytical markers differ, both studies point toward impaired systemic redox buffering in malignant ovarian disease.

Progressive rise in lipid peroxidation as shown by increasing MDA was also seen in three groups. The rise was less than that for HE4 or CA-125 and the AUC for MDA was lower when malignant illness was compared directly with benign ovarian tumors. This is biologically feasible as MDA is a non-specific result of lipid oxidation and can be raised in a wide variety of inflammatory, metabolic and chronic illness states. But the direction of change is very consistent with past ovarian cancer investigations. Ramadan et al. reported considerably higher levels of MDA and decreased activity of antioxidant enzymes in epithelial ovarian cancer and MDA showed a positive correlation with tumor stage. Redox disturbance was particularly prominent in poorly differentiated grade 3 tumors, and Caglayan et al. also documented enhanced lipid peroxidation in epithelial ovarian cancer [30]. These findings are highly relevant to the present malignant group, which was specifically composed of grade 3 cases. Earlier work by Bukhari et al. also found increased TOS and MDA, reduced total antioxidant status, and positive relationships between oxidative stress and CA-125 in ovarian cancer [31].

The pattern of correlations gives a handy integrated perspective of the data. In the control-plus-benign model, HE4 and CA-125 were favorably linked with TOS, MDA and OSI and negatively with TAC. The same trends were more evident in the control-plus-malignant model, with high positive coefficients for HE4 and CA-125 with oxidant markers and strong negative correlations with TAC. This strengthening is in line with the increased gap between healthy and malignant participants. It also corroborates the idea that the tumor-marker and redox domains are not independent at the group level. TOS and oxidative damage were positively associated with CA-125, as previously observed by Bukhari et al [32], while current mechanistic literature describes multiple links between reactive oxygen species, inflammatory pathways, altered iron metabolism, lipid peroxidation, and ovarian tumor progression [28]. The very strong relationship between TOS and OSI in the present analysis is expected because OSI is mathematically derived from TOS and TAC and should not be interpreted as an independent biological marker.

Very large connections were found for the tumor-marker cut-offs, especially CA-125 and HE4, and significant associations for OSI and low TAC, by odds-ratio analysis. The large estimates are a result of the good separation of

the group in the summary data. Their direction is consistent with the ROC results and published data suggesting HE4 and CA-125 are useful in differentiating malignant from benign ovarian cancers [23, 33]. However, actual interpretation of each individual cut-off must take into account disease stage, menopausal status, histologic subtype, assay platform and concurrent disorders. For instance, HE4 may be affected by renal function and age, while CA-125 may be raised in a number of benign gynecologic or inflammatory conditions. However, some of this confounding is mitigated, although not entirely, by the increased biological variability of clinical practice, even with removal of renal failure and other severe chronic conditions.

In general, malignant ovarian illness was related with higher levels of HE4 and CA-125, increased oxidative stress and decreased antioxidant capacity. The findings are consistent with modern biomarker reviews favoring combined rather than isolated information from the laboratory [2,8] and modern redox studies indicating that oxidative stress is very integrated in ovarian-cancer biology [29]. The diagnostic strength shown for HE4 and CA-125 in this dataset is not surprising for stage III, grade 3 disease, however the oxidative markers seem to offer complementary information on the metabolic milieu rather than the same level of tumor specificity. Future study with individual-level data, larger cohorts, histologic stratification, adjustment for renal function, and external validation will be needed to evaluate if the oxidative panel has independent diagnostic or prognostic value beyond HE4 and CA-125. However, the gradual stepwise changes in control, benign and malignant groups within the framework of the present analysis give a coherent biochemical picture of increasing tumor burden with escalating oxidative imbalance.

5. Conclusion

The present investigation clearly shows a biochemical trend from healthy status to benign ovarian illness and advanced ovarian cancer. HE4 and CA-125 rose significantly among the three groups and exhibited strong diagnostic differentiation, especially between malignant and benign illness. Concurrently, TOS, MDA and OSI increased while TAC decreased indicating a progressive transition to systemic oxidative stress. The high connections between tumor markers and redox measures show that tumor burden and oxidative imbalance may be developed in tandem. While HE4 and CA-125 were the best diagnostic markers in our data set, OSI and other oxidative-stress tests may provide further information on the metabolic milieu of ovarian illness. Larger investigations incorporating multiple stages and histological subtypes are needed to corroborate these findings.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

Informed Consent

Informed consent was obtained from all participants prior to their inclusion in the study. Participants were informed about the purpose of the research, and the confidentiality of their personal and clinical information was maintained throughout the study.

Data Availability Statement

The data generated and analyzed during the present study are available from the corresponding author upon reasonable request.

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