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# Seroimmunological Assay of Cancer Antigen 125 and Human Epididymis 4 During Progression-Free Survival Period in Ovarian Cancer Patients

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**ABSTRACT**

**Background & Objective:** Radical surgery is the most effective treatment in ovarian cancer advanced stages, making reliable biomarkers essential for enhancing diagnostic accuracy. Human Epididymis 4 (HE4) is considered a dependable diagnostic biomarker; its diagnostic precision is outweighed by Cancer Antigen 125 (CA 125). The present study was conducted with aim to evaluate immunoassay of CA 125 and HE4 during progression-free survival (PFS) period in ovarian cancer patients.

**Materials & Methods:** This observational analytical study was designed to answer whether the measurement of the tumour biomarkers, CA125 and HE4 protein, can aid in monitoring ovarian cancer patients in the postoperative period or detect the progression-free survival period. Seventy-three ovarian serous adenocarcinoma patients undergoing debulking surgery were grouped according to ovarian cancer grade, FIGO staging and progression free survival period during adjuvant therapy, each three months comparison of the serum level of CA125 and HE4 consequently. The study continued for about four years with various periods for each patient.

**Results:** During the PFS period, the lowest biomarkers mean of CA125 was 17.1 U/ml during the ninth month after surgery; while the mean for HE4 was 28.4 pg /ml during the sixth month. Both biomarkers increased during the PFS periods, with HE4 increasing in the ninth month and CA125 in the 12$^{th}$ month, indicating that HE4 increased before CA125. There is an increase in the mean serum level of both HE4 and CA125 with decrease in histopathological differentiation and increased FIGO staging, before surgery, two weeks after surgery, PFS period, and after PFS period.

**Conclusion:** The study found that the immunoassay of CA 125 and HE4 in ovarian cancer tissues can be used to monitor the effect of surgery and to evaluate the patient's in PFS period.

**Keywords:** Ovarian Cancer, Cancer Antigens 125 (CA125), Human Epididymis 4 (HE4), Progression-free Survival (PFS), Seroimmunological Test

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## 1. Introduction

Among gynecological malignancies, ovarian cancer has the greatest death rate, mostly because it is difficult to identify in its early stages. In advanced stages, debulking surgery is the best therapy option, thus it's crucial to have solid biomarkers to improve the accuracy of diagnoses. A reliable diagnostic biomarker, Human Epididymis 4 (HE4) is surpassed in diagnostic accuracy by Cancer Antigen 125 (CA 125). Laparoscopic biopsy improves the sensitivity and specificity of both indicators when used in conjunction with serum levels for early detection. It is necessary to assess the relevance of CA 125 and HE4 levels before and during the surgery for improved disease management since there is a lack of evidence about these markers in patients with ovarian cancer **(1-5)**. The therapeutic significance of multisequential CA 125 and HE4 results is particularly strong when it comes to tracking the evolution of ovarian cancer during treatment and post-therapy follow-up **(6-8)**. Current suggested biomarkers for disease development and therapy efficacy monitoring include HE4 and CA125 **(9, 10)**. You may stratify customized ovarian cancer patients using HE4, which is an escape from CA 125 cut-off oscillations, unless HE4 levels are elevated, even in healthy individuals with the highest CA 125 readings, there is usually little clinical significance **(11-14)**. Patients with advanced ovarian cancer are closely predicted by CA125 and HE4 levels, which are greater in patients with higher levels at first diagnosis compared to those with HE4 levels below 70 pmol/L **(15-17)**.

A crucial objective considered by both drug researchers and treating doctors as proof of a successful therapy in cancer treatment is progression-free survival (PFS), which is the amount of time patients are kept alive with their tumor static or reducing in size. The time to determine progression-free survival (PFS) starts on the first day of therapy and ends on the last day of treatment or the participant's death **(18-20)**. The present study was conducted with aim to evaluate immunoassay of CA 125 and HE4 during progression-free survival (PFS) period in ovarian cancer patients.

## 2. Materials and Methods

This observational analytical study was conducted to determine whether measuring the tumor biomarkers, CA125 and HE4 proteins, can assist in monitoring ovarian cancer patients during the postoperative period or in detecting the progression-free survival period. The levels of CA125 and HE4 protein were measured each three months (Figure 1) in ovarian cancer patients and categorizing them into different groups based on their grading, staging (International Federation of Gynecology and Obstetrics System) (FIGO), and progression-free survival periods **(21-23)**. Data collection for each patient was performed over a period of approximately four years from May 2020 to September 2024. The study was conducted in the Al-Jawad Oncology Centre in Al-Kadimain Medical City (KMC)/Baghdad/Iraq. The end of the PFS period and change to an alternative chemotherapy regime were detected by the professional oncologist team, who were alarmed by the serological assay for further investigation indication.

The target population of this study consisted of 73 ovarian cancer patients undergoing debulking surgery in Al-Kadimain Medical City or another private hospital in Baghdad. Neoadjuvant chemotherapy consisting of four cycles was administered to the patients with advanced ovarian cancer to diminish tumor growth and enhance surgical results. All patients were managed by a team of healthcare specialists, including oncologists, surgeons, nutritionists, and psychologists, to deliver complete management of symptoms and side effects, psychosocial support, four cycles of chemotherapy, and pain management. Modest physical exercise as tolerated was suggested to sustain strength and enhance healing results. The procedure was designed meticulously, generally for a few weeks following the final chemotherapy cycle, to facilitate recuperation and enhance the patient's condition. Consistent postoperative follow-up and monitoring every 1-3 months during the first year, and every 3-6 months afterward, are essential for the early discovery of recurrence, which can substantially affect treatment options and results. Patients were informed about the significance of complying with follow-up appointments and notifying their healthcare providers of any new symptoms during each visit when CA 125 and HE4 were tested (Table 2).

The patients with serous adenocarcinoma were divided into three groups based on the grade (type of tumour histopathology): Group One had a well-differentiated tumour, Group Two had a moderately differentiated tumour, and Group 3 had a poorly differentiated tumour. The patients were placed into groups based on the FIGO staging (International Federation of Gynaecology and Obstetrics) of ovarian cancer after surgery: Stage I: tumour is only in the ovaries, Stage II: tumour in one or both ovaries that have spread to the pelvis, Stage III: tumour spreading to the peritoneum outside the pelvis and/or lymph nodes, and Stage IV: metastases in other parts of the body, like the liver or pleura **(24-26)**. The inclusion criteria were ovarian cancer patients who underwent debulking surgery during the study period and had not previously received chemotherapy treatment. The exclusion criteria were patients who had undergone surgery prior to the study and had initiated chemotherapy prior to this period.

The study variables included the age of the patients, four groups according to the staging FIGO groups, and three groups according to the grades of the tumour (in addition to the calculation of CA 125 and HE4 serum levels with enzyme-linked immunosorbent assay ELISA). Follow up was done with the Taxol (Paclitaxel), Carbo (Carboplatin), and Avastine (Bevacizumab) regimen before surgery, two weeks after surgery, and then every three months during treatment. Adjuvant chemotherapy for ovarian cancer consisted of this regimen until the end of the progression-free survival period, after which it switches to a different regimen. The treatment types were prescribed by a professional oncologist doctor team in Al-Jawad Oncology Centre and are not included as variables in the treatment.

**Enzyme-Linked Immunosorbent Assay (ELISA) test methodology**

The sandwich quantitative two-step colorimetric ELISA test with two different antibodies was used to identify the CA125 and HE4 antigens. The manufacturer directed each step differently to detect the Human Carbohydrate Antigen 125/MMucin-16 ELISA Kit from Sigma-Aldrich Chemie GmbH, Product Number: RAB0376. When the sample was prepared by gathering serum, specimens and standards (provided by the manufacturer) should be added to wells coated with a CA125 antibody. Then allow the CA125 antibody to bind to the antibody. After that, an enzyme-conjugated secondary antibody should be added to the wells to see if the CA 125 antigen/antibody complex is there. This indicates the presence of CA 125 in the patient's serum sample. Finally, substrate should be added after the washing step to create a measurable color change. A spectrophotometer can be used to measure the optical density (OD) at a specific wavelength specified by the protocol and correlate it with the CA125 concentration. Adherent should be performed to the specific protocol

that comes with the kit for information on reagent volumes and incubation times. The same steps above were used to detect the HE4 antigen in the patient's serum, using a Human HE4/WFDC2/WAP5 ELISA Kit (catalogue number: kit12609) from Sino-Biological Company. To enhance precision and prevent bias, a portion of the serum under examination were stored in a deep freeze. This allows us to repeat the measurements for CA 125 or HE4, minimizing potential sources of bias during data calculation and investigation **(14, 27, 28)**. All ovarian cancer cases who met the inclusion criteria were included in this study.

Data were analyzed using IBM SPSS statistics (version 24), and using the chi-square test, ANOVA test, test of homogeneity of variances, Spearman test, likelihood ratio.

### Ethical Considerations

The study has no effect on the treatment plan that was decided via a professional oncology doctor's team according to each patient's situation. The study primarily relies on the decisions made by the professional oncology doctor at the Al-Jawad Oncology Centre regarding adjuvant therapy for postoperative OC patients. The study covered the financial costs, including the seroimmunological assay, and had no connection to the patient's treatment costs. The study received ethical approval from Al-Jawad Oncology Centre and other private centers.

## 3. Results

During the PFS period after surgery, the lowest biomarker mean for CA125 was 17.1 U/ml during the ninth month, while the mean for HE4 was 28.4 pg /ml during the sixth month. Both biomarkers increased during the PFS periods, with HE4 increasing in the ninth month and CA125 in the 12th month, indicating that HE4 increased before CA125 (Figure 1) (Table 2).

The majority of ovarian cancer patients (54.7%), with an average age of 61.3 years, with a mean serum level of 1434.3 U/ml for CA125 and 767 pg/ml for HE4 received a well-differentiated grading. After two weeks of surgery, the mean values for CA125 and HE4 were 17 U/ml and 33.2 pg/ml, respectively. The mean PFS period for this group was 15.9 months, and the mean FIGO staging was 3.1. The moderately differentiated group, comprising 12 patients, represented 17.14% of the total patients, with an average age of 58.33 years. The mean CA125 level before surgery was 1373.3 U/ml, while the mean HE4 level was 549.3 pg/ml. After two weeks of surgery, the mean CA125 level was 23.3 U/ml and the mean HE4 level was 39 pg/ml. After PFS period, the mean of CA125 was 174.3 U/ml and for HE4 it was 326 pg/ml. In the poorly differentiated group before surgery, the mean CA125 level was 3153.8 U/ml and the average HE4 level was 821.3.3 pg/ml. Two weeks after surgery, the average CA125 level was 16.3 U/ml and the average HE4 level was 45.7 pg/ml. After the PFS period, the average CA125 level was 277.1 U/ml and the average HE4 level was 331.1 pg/ml. The average PFS for this group was 21.05 months, but the average FIGO stage for this group was 2.3. There was a significant correlation between the levels of HE4 and CA125 in the patient's serum and the different histopathological grading (Table 1).

**Table 1. The main variables of the study are associated with the histopathological differentiation of the ovarian cancer tissue**

| Ovarian Cancer types and Grades | | Age of Patients | CA 125 Before Surgery | CA 125 After Surgery | CA 125 After PFS | HE4 Before surgery | HE4 After Surgery | HE4 After PFS | (FIGO) staging | PFS |
|---|---|---|---|---|---|---|---|---|---|---|
| Grade 1 | Mean | 61.3 | 1434.3 | 17 | 113.5 | 767 | 33.3 | 324.7 | 3.1 | 15.9 |
| | N | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| | Std. Deviation | 13.17 | 1496.7 | 10.73 | 52.48 | 265.35 | 9.6 | 60.69 | .95 | 4.9 |
| | Minimum | 42 | 487 | 5.00 | 63 | 263 | 21 | 250 | 1 | 12 |
| | Maximum | 76 | 5284 | 36 | 23 | 1295 | 54 | 430 | 4 | 30 |
| Grade 2 | Mean | 58.33 | 1373.3 | 23.33 | 174.3 | 549.33 | 39 | 326 | 1.7 | 21.3 |
| | N | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Std. Deviation | 17.94 | 642.14 | 1.78 | 44.09 | 338.67 | 17.2 | 49.55 | .985 | 6.9 |
| | Minimum | 38 | 550 | 21. | 143 | 243 | 22 | 270 | 1 | 12 |
| | Maximum | 80 | 2027 | 25 | 234 | 998 | 70 | 386 | 3 | 27 |
| Grade 3 | Mean | 53.43 | 3153.77 | 16.33 | 277.1 | 821.33 | 45.71 | 331.1 | 2.3 | 21.1 |
| | N | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |

| | Ovarian Cancer types and Grades | Age of Patients | CA 125 Before Surgery | CA 125 After Surgery | CA 125 After PFS | HE4 Before surgery | HE4 After Surgery | HE4 After PFS | (FIGO) staging | PFS |
|---|---|---|---|---|---|---|---|---|---|---|
| Total | Std. Deviation | 3.44 | 2886.97 | 9.12 | 132.72 | 539.59 | 14.41 | 59.5 | 1.4 | 4.6 |
| | Minimum | 48 | 352 | 4 | 54 | 283 | 23 | 232 | 1 | 15 |
| | Maximum | 57 | 7100 | 30 | 432 | 1574 | 70 | 403 | 4 | 27 |
| | Mean | 58.55 | 1918.92 | 17.85 | 170.56 | 746.85 | 37.79 | 326.8 | 2.6 | 18.3 |
| | N | 73 | 73 | 73 | 73 | 73 | 73 | 73 | 73 | 73 |
| | Std. Deviation | 12.582 | 2053.39 | 9.59 | 108.64 | 380.64 | 13.51 | 57.98 | 1.2 | 5.8 |
| | Minimum | 38 | 352 | 4 | 54. | 243 | 21 | 232 | 1 | 12 |
| | Maximum | 80 | 7100 | 36 | 432 | 1574 | 70 | 430 | 4 | 30 |

This correlation led to an increase in the mean serum levels of both HE4 and CA125. This increase was observed in all periods, including before surgery, two weeks after surgery, the PFS period, and after the PFS among the 73 patients diagnosed with FIGO stage I-IV, 30.1% (n=22) had stage I disease, 6.8% (n=5) had stage II, 32.9% (n=24) had stage III, and 30.1% (n=22) had stage IV.

According to the FIGO staging grouping, (Table 2), the mean values of CA-125 were as follows: for stage I, 729.1364 U/ml before surgery, 14.3182 U/ml after 2 weeks of surgery, and 191.5 U/ml after PFS of 25.69 months. In stage II, the mean values were 558.2 U/ml before surgery, 26.2 U/ml after 2 weeks of surgery, and 91 U/ml after PFS of 16.8 months. In stage III, the mean values of CA125 was 1937.33 U/ml before surgery, 26.2 U/ml after 2 weeks of surgery, and 173.5 U/ml after PFS of 15.75 months. In stage IV, the mean values of CA125 was 3397.86 U/ml before surgery, 22.41 U/ml after 2 weeks of surgery, and 164.5 U/ml after PFS of 13.91 months.

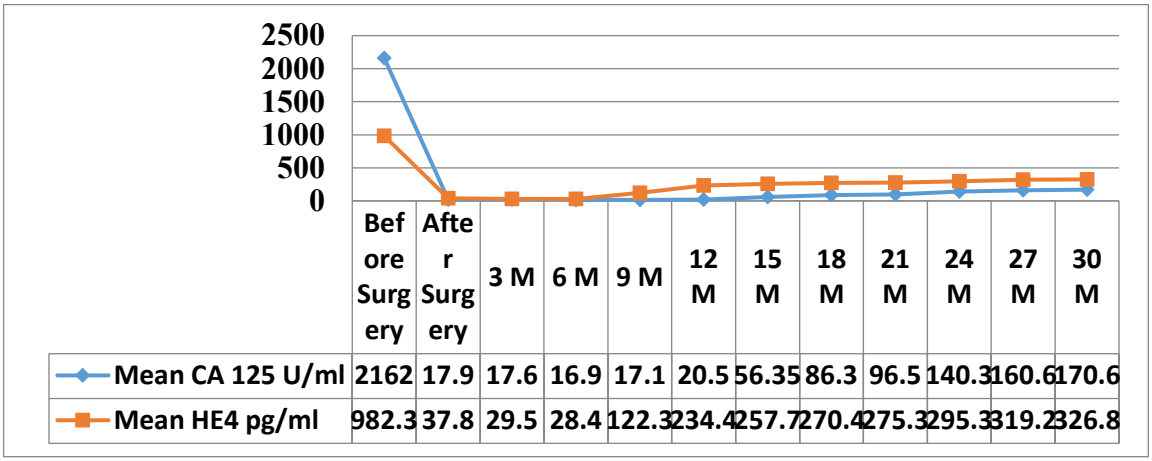



**Figure 1. The mean CA125 and HE4 in correlation to the PFS periods in ovarian cancer patients.**

There was a significant correlation between the levels of CA125 and HE4 in patients with the FIGO staging before surgery (P< 0005), as well as after the PFS period (P < 05). This was due to an increase in the mean serum level of CA125 and HE4 in patients with high FIGO staging, whereas there was no significant correlation in the two weeks following the surgery.

According to the FIGO staging grouping, the mean values of HE4 for stage I was 311 pg/ml before surgery, 37.19 pg/ml after 2 weeks of surgery, and 346.73 after PFS. The mean values of HE4 for stage II was 532.4 pg/ml before surgery, 28 pg/ml after 2 weeks of surgery, and 257 pg/ml after PFS.

**Table 2. The mean values and time intervals for the assessment of CA125 in U/ml and HE4 in pg/ml for patients about PFS.**

| Duration in months for tumor markers | Before Surgery | 2 weeks After Surgery | 3 M | 6 M | 9M | 12 M | 15 M | 18 M | 21 M | 24 M | 27 M | 30 M | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean CA125 U/ml** | 2161.81 | 17.9 | 17.6 | 16.9 | 17.1 | 20.5 | 56.35 | 86.3 | 96.5 | 140.32 | 160.64 | 170.56 | |
| **Mean HE4 pg/ml** | 982.3 | 37.7945 | 29.5 | 28.4 | 122.34 | 234.4 | 257.67 | 270.43 | 275.34 | 295.27 | 319.23 | 326.75 | |
| **Patient distribution according to the PFS** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 14 | 2 | 9 | 11 | 2 | 73 |

The mean values of HE4 for stage III were 873.83 pg/ml before surgery, 42.25 pg/ml after 2 weeks of surgery, and 331.67 pg/ml after PFS; for stage IV, the mean values of HE4 were 1092.91 pg/ml before surgery, 35.77 pg/ml after 2 weeks of surgery, and 317.23 pg/ml after PFS. There was a substantial association between the levels of HE4 in patients with the FIGO staging before surgery ($P < 0005$) and after the PFS period ($P < 05$), as there was an increase in the mean serum level of HE4 in high FIGO staging. However, no significant association was observed in the two weeks post-operative period.

**Table 3. The association between the FIGO staging regarding CA125 and HE4 levels in ovarian cancer patients**

| | FIGO Stage | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| **Age of Patients** | **I** | 22 | 48.364 | 7.22130 | 45.1619 | 51.5654 | 38 | 57 |
| | **II** | 5 | 71.4 | 10.28591 | 58.6284 | 84.1716 | 53 | 76 |
| | **III** | 24 | 62.333 | 11.79044 | 57.3547 | 67.3120 | 47 | 80 |
| | **IV** | 22 | 61.682 | 11.98168 | 56.3694 | 66.9942 | 43 | 74 |
| | **Total** | 73 | 58.548 | 12.58200 | 55.6123 | 61.4835 | 38 | 80 |
| **CA125 Before Surgery** | **I** | 22 | 729.17 | 410.0351 | 547.337 | 910.936 | 352 | 1543 |
| | **II** | 5 | 558.2 | 159.208 | 360.517 | 755.88 | 487 | 843 |
| | **III** | 24 | 1937.3 | 1762.965 | 1192.9 | 2681.77 | 650 | 5600 |
| | **IV** | 22 | 3397.9 | 2581.391 | 2253.34 | 4542.39 | 520 | 7100 |
| | **Total** | 73 | 1918.9 | 2053.389 | 1439.83 | 2398 | 352 | 7100 |
| **CA 125 After Surgery** | **I** | 22 | 14.318 | 8.59339 | 10.5081 | 18.1283 | 4 | 25 |
| | **II** | 5 | 26.2 | 6.26099 | 18.4260 | 33.9740 | 21 | 34 |
| | **III** | 24 | 15.167 | 9.26971 | 11.2524 | 19.0809 | 5 | 30 |
| | **IV** | 22 | 22.409 | 8.96373 | 18.4348 | 26.3834 | 12.00 | 36.00 |
| | **Total** | 73 | 17.849 | 9.59191 | 15.6114 | 20.0873 | 4.00 | 36.00 |
| **CA 125 After PFS** | **I** | 22 | 191.5 | 104.5752 | 145.134 | 237.866 | 54.00 | 354 |
| | **II** | 5 | 91 | 2.23607 | 88.2236 | 93.7764 | 90.00 | 95.00 |
| | **III** | 24 | 173.5 | 95.16073 | 133.317 | 213.683 | 65.00 | 345 |
| | **IV** | 22 | 164.5 | 132.6613 | 105.681 | 223.319 | 76 | 432 |
| | **Total** | 73 | 170.56 | 108.6434 | 145.213 | 195.910 | 54.00 | 432 |
| **HE4 Before Surgery** | **I** | 22 | 311 | 60.40498 | 284.218 | 337.782 | 243 | 407 |
| | **II** | 5 | 532.4 | 5.36656 | 525.737 | 539.064 | 530 | 542 |
| | **III** | 24 | 873.83 | 225.1341 | 778.768 | 968.899 | 549 | 1256 |
| | **IV** | 22 | 1092.9 | 289.3572 | 964.615 | 1221.20 | 835 | 1574. |

| | FIGO Stage | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| | **Total** | 73 | 746.85 | 380.6356 | 658.04 | 835.658 | 243 | 1574. |
| **HE4 After Surgery** | **I** | 22 | 37.182 | 12.08555 | 31.8234 | 42.5403 | 22 | 62 |
| | **II** | 5 | 28 | 9.05539 | 16.7563 | 39.2437 | 21 | 43 |
| | **III** | 24 | 42.25 | 16.11710 | 35.4443 | 49.0557 | 21.00 | 70.00 |
| | **IV** | 22 | 35.773 | 11.35543 | 30.7380 | 40.8074 | 21.00 | 70.00 |
| | **Total** | 73 | 37.795 | 13.50510 | 34.6435 | 40.9455 | 21.00 | 70.00 |
| **HE4 After PFS** | **I** | 22 | 346.77 | 50.44746 | 324.406 | 369.14 | 232 | 403 |
| | **II** | 5 | 257 | 15.65248 | 237.565 | 276.435 | 250 | 285 |
| | **III** | 24 | 331.67 | 59.41356 | 306.579 | 356.755 | 270 | 430 |
| | **IV** | 22 | 317.23 | 58.01965 | 291.503 | 342.952 | 255 | 420 |
| | **Total** | 73 | 326.75 | 57.97815 | 313.226 | 340.281 | 232 | 430 |
| **PFS** | **I** | 22 | 25.682 | 2.71480 | 24.4781 | 26.8855 | 18.00 | 30.00 |
| | **II** | 5 | 16.800 | 4.02492 | 11.8024 | 21.7976 | 15.00 | 24.00 |
| | **III** | 24 | 15.750 | 2.21163 | 14.8161 | 16.6839 | 12.00 | 18.00 |
| | **IV** | 22 | 13.909 | 3.53094 | 12.3436 | 15.4746 | 12.00 | 24.00 |
| | **Total** | 73 | 18.260 | 5.75429 | 16.9177 | 19.6028 | 12.00 | 30.00 |

## 4. Discussion

The markers like CA 125 and HE4 are used to monitor the development of ovarian cancer and the effectiveness of treatment. Physicians have proposed the use of CA 125 and HE4 as multimarker diagnostic biomarkers for managing ovarian cancer. The candidate HE4 marker is very different from normal mucosa and benign epithelium/adenomas; it also differentiates absolute cancer cells better than any other marker. Studies have shown that high levels of both HE4 and CA 125 can help find women who are more likely to have a malignant pelvic mass and makes the diagnosis more accurate when used with CA 125 **(25)**.

Patients are grouped based on the FIGO staging and histopathological grading (ASCO 2016) **(24)**. The progression-free survival (PFS) period is used to detect any changes in the patient's serum levels for CA125 and HE4. This study focuses on the ability of CA125 and HE4 markers to predict progressive disease or the PFS period when used together in serial measurements. This underscores the importance of evaluating the significance of CA125 and HE4 levels before and after surgery, as well as until the end of the progression-free survival periods, to improve disease management.

The goal of gynecological oncologists is to develop a new surgical technique for primary and recurrent ovarian cancer patients. Thus, searching for new biochemical markers for the management of these patients is a priority for developing personalized treatment in oncology **(23, 29)**. This study focuses on the potential role of established CA125 and HE4 together as immunological assay procedures for initial ovarian cancer diagnosis and also for detection of early tumor relapse referral. This study assessed ovarian cancer patients based on two categories: the FIGO staging, which defines the extension of the disease, and the grading, which evaluates the tumor aggressiveness. The correct oncologic management of ovarian cancer is directly linked to a precise evaluation of all aspects of the ovarian neoplasm in order to customize the best individual therapeutic strategy, so we attempt to study the effect of the staging and grading variance with the serum level of HE4 and CA125 during pre-surgical, post-surgical and adjuvant therapy period (PFS) **(6, 30)**.

There is limited data regarding CA125 and HE4 markers in ovarian cancer patients, illustrating the need to evaluate the expressive of CA125 and HE4 before and subsequently two weeks after surgery to better manage the disease **(14, 15)**. In the present study, during the PFS period, the mean HE4 started to increase during the nine months, while CA125 during the 12$^{th}$ month, that to say HE4 increased before the CA125. While HE4 is considered a dependable biomarker, its diagnostic precision is outweighed by CA125 (1). The arrangement of the CA125 with HE4 immunoassay for ovarian cancer diagnosis had 98% specificity. Additionally, the combination of CA125 and HE4 is prognostic for recurrence, and monitoring their levels throughout the disease and after

chemotherapy may serve as a surrogate for survival **(2, 7, 31)**.

In the first few weeks after the radical ovarian cancer surgery, the CA125 or HE4 levels initially drop rapidly, but they cannot be used to determine residual disease status. Both levels of these two biomarkers are influenced by ongoing surgical and postoperative factors such as sub-diaphragmatic manipulation with subsequent release of tumor cells, hemodilution, and other hemodynamic changes, local trauma at surgery **(4, 8, 32)**. In the current research, 40 patients out of the 73 ovarian cancer patients were diagnosed with the well-differentiated grading. However, the PFS was the lowest mean period, 15.9 months, if compared with another grade; that may be due to most of these cases being a late diagnosis in stage 3 or more (mean FIGO staging 3.1). In the moderately differentiated group (12 patients), the mean PFS period was 21.25 months. However, most of them were diagnosed in the early FIGO staging (mean staging 1.67), so they have the highest PFS period. The last group of poorly or undifferentiated ovarian cancer patients represented 21 patients with a relatively good PFS period of 21.1 months, which can be related to most of the patients being in early FIGO stages with a mean stage of 2.29. The findings of the present study indicate the great effect of FIGO staging on the PFS period; at the same time, there is no clear answer about the effectiveness of CA125 and HE4 markers in the quality staging of the disease and in the prognosis of verification of ovarian cancer patients **(6, 14, 33)**. In FIGO, ovarian cancer is classified as operable if the tumor has no dramatic spread. Accordingly, the use of preoperative tumor markers and visualization methods will allow the identification of ovarian cancer patients at the level of preoperative ovarian cancer for the safety and security of surgical interventions. The researchers reported that the use of ovarian cancer markers to predict the presence of persistent disease in patients with optimal tumor resection after the third postoperative week can be a determining factor in making decisions about the use of surgery in the primary stage of ovarian cancer **(16, 34)**.

## 5. Conclusion

A combination of CA125 and HE4 tumor markers have still been widely used for diagnosis combined with imaging methods. In addition to diagnosis, the study found that the expression of HE4 and CA125 in ovarian cancer tissues can be used to monitor the effect of surgery. It may even use to evaluate the patient's prognosis after surgery. However, due to cost restrictions for other non-invasive investigations, it has been widely validated in clinical applications. However, it still needs more evaluation in depth since it can be detected in human blood before and after ovarian cancer surgery. More analysis is needed regarding the relationship between CA125 and HE4, as well as prognostic factors such as staging, tumor grading, and the patient disposition to obtain complete or incomplete radical surgical tumor removal, adjuvant therapy, and PFS period. This higher negative predictive has been described in the last few years for both markers and can be easily explained. When both CA125 and HE4 are very high in OC patients, then it is certain that the disease is well advanced, macroscopic peritoneal spreading is already present, and capillaries have been invaded, above all in the patients not submitted to adjuvant treatments.

## 6. Declarations


### Acknowledgments

The authors would like to thank the staff of the Al-Jawad Oncology Centre in Al-Kadimain Medical City (KMC)/Baghdad/Iraq.


### Ethical Considerations

The study does not influence the treatment plan established by a team of trained oncologists based on each patient's circumstances. The research predominantly depends on the determinations of the oncologist at the Al-Jawad Oncology Centre concerning adjuvant therapy for postoperative ovarian cancer patients. The study addressed the financial expenditures, encompassing the seroimmunological assay, and was unrelated to the patient's treatment expenses. The research obtained ethical clearance from Al-Jawad Oncology Centre and additional private institutions.

### Authors' Contributions

1. Conceptualization: Formulating the research idea encompassing all researchers involved. 2. Data curation: Gathering, monitoring, and managing all clinical information pertaining to patients at Al-Jawad Oncology Center, as well as organizing and sanitizing the clinical data in collaboration with Hawraa Abdulameer and Yusra Jabbar Hasan.

3. Formal Analysis: Methodology of Study Design Formulating or modifying research methodologies, protocols, or processes Executing experiments, surveys, or other forms of inquiry. Conducting statistical analysis, modifying software utilized in research data modeling, seroimmunological, and other laboratory information and formal analyses by Abdulameer Kareem. Leelo Al-Obaidy.

### Conflict of Interest

The authors declare that there are no conflicts of interest.


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