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Utilising of CA125 As a Basic Factor To Differentiate Between Serous And Mucinous Carcinomas In Ovarian Patients Cancer In Basrah

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Abstract

Background: Early detection of endometrial cancer improves survival. Ovarian cancer is the most lethal form of cancer affecting the female reproductive system and ranks as the fifth highest cause of mortality among women. The CA-125 test is a crucial diagnostic tool for detecting ovarian cancer. Ovarian tumours lacking CA125 expression may exhibit heightened sensitivity to serum cancer markers, facilitating early detection. Accuracy and precision The sensitivity of CA125 for early-stage ovarian cancer identification ranges from 50% to 62% due to the fact that only half of the patients have elevated CA125 levels. A potential diagnosis of ovarian cancer may include elevated levels of CA125, sometimes referred to as cancer antigen 125, While CA125 is not the primary diagnostic tool for ovarian cancer, it is valuable for the purposes of detection and treatment. **Methods:** The study comprised the collection of (44) patient samples only out of (134) total samples. of the samples of patients with ovarian cancer . female clinically Patients were attended Al-Muana General Hospital in Basra City from March 2022 to March 2023. After laboratory diagnosis, the results of the samples of patients with ovarian cancer were. The ELISA method measured the concentration of CA 125 in serum and plasma in a controlled laboratory setting. **Results:** patients diagnosed with high-grade serous ovarian cancer (group 1) had markedly elevated blood CA125 levels ($p<0.01$), but those with mucinous carcinomas (group 2) showed lower values. Additionally, this study revealed that CA125 levels do not increase in grade 1 and 2 endometrial cancer (group 3), but they do rise in grade 3 (group 4). CA125 levels dropped in grade 1 Endometrial Endometrioid carcinoma (group 5), but rose in group 6 Endometrial carcinoma in the ovary and uterus. **Conclusions:** High-grade seromas have elevated levels of CA125 compared to mucosal malignancies. Elevated levels of CA125 can be observed in conditions such as endometriosis, pelvic inflammatory disease and other non-malignant gynecological disorders, as well as ovarian cancer. While CA125 levels can be raised in advanced stages of ovarian cancer. And they may not always accurately correlate with the extent of metastasis.

Keywords: Ovarian Carcinoma, CA125, Mucosal Malignancies , Serous Cancers

1-Introduction

Ovarian cancer is the most lethal gynecologic cancer and ranks as the fifth highest cause of mortality linked to cancer in women (1). Ovarian cancer is a prevalent gynecologic cancer that ranks third in frequency, behind cervical and uterine cancer (2). It is projected that the death rate of this malignancy will increase dramatically by the year 2040 (3). Ovarian carcinomas include a wide range of tumours that vary in kind and composition (4). They consist of distinct entities with distinct molecular characteristics, causes, precursor abnormalities, and variables that increase the likelihood of their development (5). Brenner, squamous cell, transitional cell, and undifferentiated carcinomas are examples of epithelial tumours that are associated with a lower incidence(6). Though dysgerminomas, yolk sac tumours, and immature teratomas are examples of germ cell tumours, which start from primordial germ cells, germ cell tumours are more common (7). Serous and mucinous cystadenomas, Brenner tumours, fibromas, and dermoid and dermoid cystadenomas are all examples of benign ovarian tumours(8),

CA125 is a protein that is present in higher amounts in the blood of some people, especially women, It is often used as a biomarker for ovarian cancer, tumour markers are biomolecules that exhibit elevated levels in the blood, urine, or tissues of patients afflicted with certain forms of cancer (9). CA125 is mostly linked to ovarian cancer, however it may also be raised in other disorders such as endometriosis, uterine fibroids, and pelvic inflammatory disease (10). It is crucial to acknowledge that increased *CA125* levels do not automatically imply the existence of cancer, and other criteria should be taken into account throughout the diagnosis procedure (11).

Extensive research has been conducted on several blood tumour early Detection of Ovarian Cancer biomarkers, radiographic imaging modalities, and risk stratification algorithms to achieve early detection of ovarian cancer (12). Women diagnosed with early-stage ovarian cancer often have few symptoms. In contrast, the advancement of the illness may be linked to more apparent non-specific symptoms, such as stomach bloating, discomfort, and swelling, early feeling of fullness, and frequent or urgent need to urinate, furthermore, when the condition advances, there may be a subsequent occurrence of severe weight loss and intestinal blockage (13). Early detection of ovarian cancer, especially before symptoms occur, is crucial, this is because pre symptomatic patients have a better outlook, despite their increased risk of early detection, type I tumours have better survival rates due to their molecular makeup (14). Consequently, screening for ovarian cancer in a woman without symptoms and with an average risk is now not the most efficient choice (15).

Cancer Antigen 125, a glycoprotein produced by the (*MUC16*) gene, is released from the coelomic and müllerian epithelia into the circulation (16). Ovarian cancer patients express CA125 in their blood, allowing doctors to distinguish malignant ovarian tumours from the normal population (NICE) of the UK recommended CA125 as a screening test for women with ovarian cancer symptoms (17). Postmenopausal women with CA125 levels exceeding 35 U/mL are at high risk of cancer CA125 is the best blood biomarker for ovarian cancer diagnosis due to its extensive study and application, tested 92 preselected proteins in blood samples taken fewer than 18 months before ovarian cancer discovery (10). In addition to CA125, no additional indicators provided diagnostic discrimination for ovarian cancer identified more than 9 months after the blood sample was obtained (18).

Sensitivity and specificity the sensitivity of CA125 for early-stage ovarian cancer identification is low, ranging from 50% to 62%, since only half of the patients have elevated CA125 levels (19). Advanced-stage patients were differentiated from healthy controls only based on blood CA125 levels, discovered that persons who had normal CA125 levels in the past were more likely to identify ovarian cancer at an early stage compared to those with elevated levels. Consequently, relying only on CA125 screening may result in a delay in diagnosis and a deterioration of outcomes for women (18). The specificity of CA125 is restricted, ranging from 73% to 77%. Additionally, over 60% of women with high CA125 levels do not have ovarian cancer (19). Elevated CA125 levels may be caused by several factors such as pregnancy, menstruation, and different types of malignancies including breast, uterine, stomach, pancreatic, liver, and colon cancer (20).

Additionally, benign diseases such as acute pelvic inflammation, adenomyosis, uterine myoma, and endometriosis can also lead to increased CA125 levels (21). Among the 414 Asian women we studied who had adnexal masses, 31.5% of those with non-cancerous conditions had elevated CA125 levels (22).The Prostate, lung, Colorectal, and Ovarian (*PLCO*) cancer screening trial revealed that the positive predictive value (*PPV*) for CA125 alone was just 3.7%, CA125 has limited efficacy in non-epithelial ovarian malignancies, clear cell carcinomas, undifferentiated carcinomas, and mucinous carcinomas (23). Multiple factors may influence blood CA125 levels, leading to variations in baseline values among women (10).

Importance of CA125 in the diagnosis of ovarian cancer ovarian cancer patients may have elevated blood levels of CA125, also known as cancer antigen 125(18). Surveillance and supervision CA125 is often used as a diagnostic test for the surveillance of ovarian cancer patients during the course of their therapy and post-treatment, tracking variations in CA125 levels over time may aid in evaluating the effectiveness of treatment and identifying possible recurrence (24).

Elevated CA125 levels may be seen in diseases other than ovarian cancer, including endometriosis, pelvic inflammatory disease, and some benign tumours, the absence of precise details restricts the use of CA125 as a sole diagnostic instrument (25). Restricted sensitivity and specificity elevated levels of CA125 may not be present in every instance of ovarian cancer, especially during the first phases, consequently, it lacks the ability to accurately identify early signs. CA125 levels may be influenced by several variables, including age, menopausal state, and the specific kind of ovarian cancer (26). Carcinoma specificity with a focus on seriousness Although CA125 is not completely exclusive to serous carcinomas, it is often more increased in high-grade serous ovarian carcinomas in comparison to other subtypes, this may provide further data for physicians in delineating the kind and severity of the ovarian cancer (27).

2- Methods

The study comprised the collection of (44) patient samples only out of (134) total samples. of the samples of patients with ovarian cancer . female clinically Patients were attended Al-Muana General Hospital in Basra City from March 2022 to March 2023. Approval for conducting the study was obtained from the ethical Committee of Al-Muana General Hospital and informed consent was obtained from the patients. The using ELISA method measured the concentration of CA 125 in serum and plasma in a controlled laboratory setting.

2-1 Intended

(CA 125) ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of CA 125 in serum and plasma.

2-2 Summary and explanation

The (*CA125*) ELISA is an assay for the detection of (OC125) reactive determinants on a heterogeneous (high-molecular-weight 200 - 1000 kDa glycoprotein) in serum. The (*CA 125*) monoclonal antibody developed by (Bast *et al*.,1981) who was the first to identify this glycoprotein. A substantial proportion of non-mucinous epithelial ovarian tumours include (*CA125*) reactive determinants, which are also present in the serum of women with such tumours. Most patients with active epithelial ovarian cancer, even those in stage I, have elevated (*CA125*) readings ,1-2% of healthy people have increased(*CA125*) readings, and these values may also be raised in, a variety of illnesses, both benign and malignant, besides ovarian cancer. .

A (*CA125*) test value more than or equal to 35 U/mL was discovered to be suggestive of existence of residual tumour in women with primary epithelial ovarian cancer who had received

first-line treatment followed by diagnostic second-look procedures Because patients with histopathologic evidence of ovarian carcinoma may have (CA 125) test values within the range of healthy persons, the value below 35 U/mL does not imply the absence of residual ovarian cancer.

2-3 Principle of the test

(*CA 125*) ELISA - kit is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle the microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the CA 125 molecule. An aliquot of patient sample containing endogenous CA 125 is incubated in the coated well with enzyme conjugate, which is a monoclonal anti- (*CA 125* antibody) conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off, the amount of bound peroxidase is proportional to the concentration of CA 125 in the sample. having added the substrate solution, the intensity of colour developed is proportional to the concentration of CA 125 in the patient sample.

2-4 Reagents provided

The reagents required and quantity used for determinate (CA125) marker in all specimens showed in Table (2-1) .

Reagents provided TM E-4331 Microtiter wells 12x8 (break apart) strips, 96 wells: Wells coated with anti-CA 125 antibody (monoclonal) , Standards + Controls:

Table (2- 1): Standards and controls used for CA125 determination

at. no.	**tandard**	**oncertation**	**olume/Vial**
M E-4301	ero Standard	U/mL	ml
M E-4302	tandard 1	5 U/mL	.5 ml
M E-4303	tandard 2	5 U/mL	.5 ml
M E-4304	tandard 3	50 U/mL	.5 ml
M E-4305	tandard 4	00 U/mL	.5 ml
M E-4306	tandard 5	00 U/mL	.5 ml
M E-4351	ontrol 1	or control values and nges	.5 ml
M E-4352	ontrol 2	efer to vial label of QC-ata sheet	.5 ml

Conjugate 1 vial, 7 mL, ready to use, Anti-CA 125 antibody (monoclonal) conjugated to horseradish peroxidase; Contains non-mercury preservative.

Substrate 1 vial, 14 mL, ready to use, TMB.

Stop-solution 1 vial, 14 mL, ready to use; contains 0.5M H2SO4. Avoid contact with the stop solution. It may cause skin irritations and burns.

Wash-solution 1 vial, 30 mL (40X concentrated) , Preparation of Reagents.

Wash-solution 1 vial, 30 mL (40X concentrated) , Preparation of Reagents.

2-5 Materials required

-Microplate (A microtiter plate calibrated reader (450 ± 10 nm))

_Single or multi-channel pipettes with high precision and disposable tips

-Micro centrifuge Tubes

-Deionized or distilled water

-Absorbent paper for blotting the microplate

-Container for Wash Solution

-Graph paper or software for data reduction

_unopened reagents will remain reactive up to their expiration date when kept at 2 °C to 8 °C. Reagents should not be used after this time.

_Opened reagents must be kept between (2 and 8°C). The recommended storage range for microtiter wells is (2 to 8 °C). After the foil bag has been opened, it must be carefully sealed shut once more. Opened kits can stay active for 8 weeks if kept according to the instructions above.

. .

Prepare the Reagents

Before using, acclimatise the necessary quantity of strips and reagents to room temperature, then deionized water was added to the (40X) concentrated wash Solution. Dilute (30 ml) of concentrated wash solution with (1170 ml) deionized water to a final volume of (1200 ml) .

2-5 Specimen preparation and storage

After blood collection with serum separator tube, samples allowed to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1,000×g , centrifugation was completed after clotting has occurred. Assay freshly prepared serum immediately or store samples in aliquot at (-20℃ or -80℃) for later use and avoid repeated freeze /thaw cycles. Specimens should be capped and may be stored for up to 2 days at(2 °C - 8 °C), prior to assaying

specimens held for a longer time (up to 12 months) should be frozen only once at(-20°C)prior to assay.

2-6 Calculation

1- Average absorbance values should be calculated for each set of standards, controls, and patient samples.

2- The mean absorbance obtained from each standard is plotted against its concentration using the linear graph paper, with the absorbance value on the vertical (Y) axis and the concentration on the horizontal (X) axis.

3- Determine the appropriate concentration from the standard curve using the mean absorbance value for each sample.

4- Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter). Logistics curve fit. 4 Parameter Logistics is the preferred method .Other data reduction functions may give slightly different results.

5- The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as 600< U/Ml.

2-7 Procedure

Each run must include a standard curve

1-Secure the desired number of Microtiter wells in the frame holder

2-Dispense 50 µl of each Standard, Control and samples with new disposable tips into appropriate well.

3-Dispense 50 µl Enzyme Conjugate into each well.

Thoroughly mix for (10 seconds). It is important to have a complete mixing in this step.

4- Incubate for (60 minutes) at room temperature

5- Briskly shake out the contents of the wells strike. the wells sharply on. Rinse the wells 3 times with diluted wash solution (300 µl per well), remove residual droplets by absorbent paper.

Note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure.

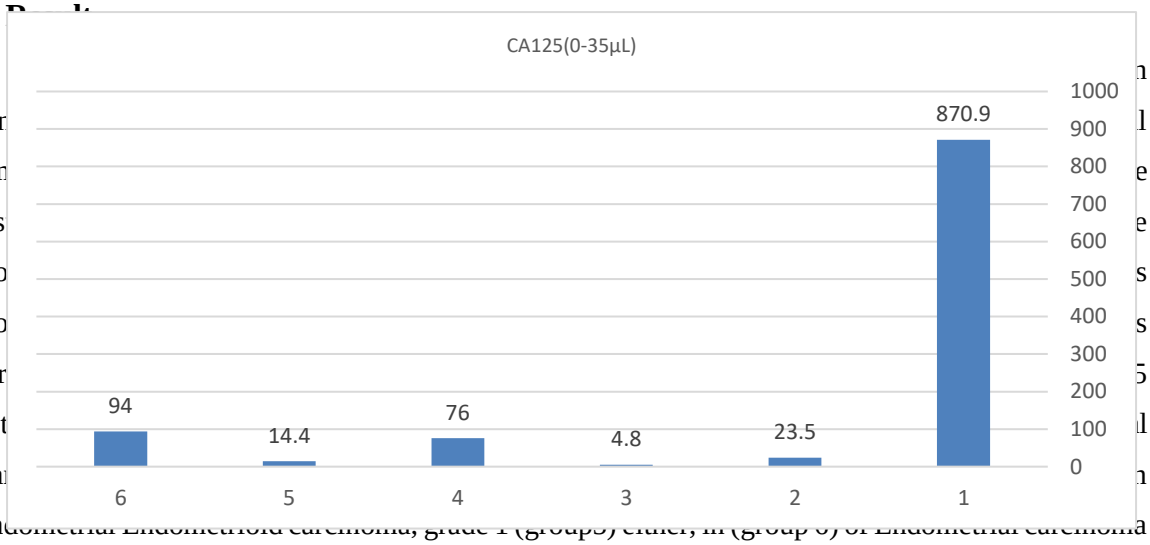
6- Added (100µl) of substrate solution to each well .

7- Incubate for (15 minutes) at room temperature.

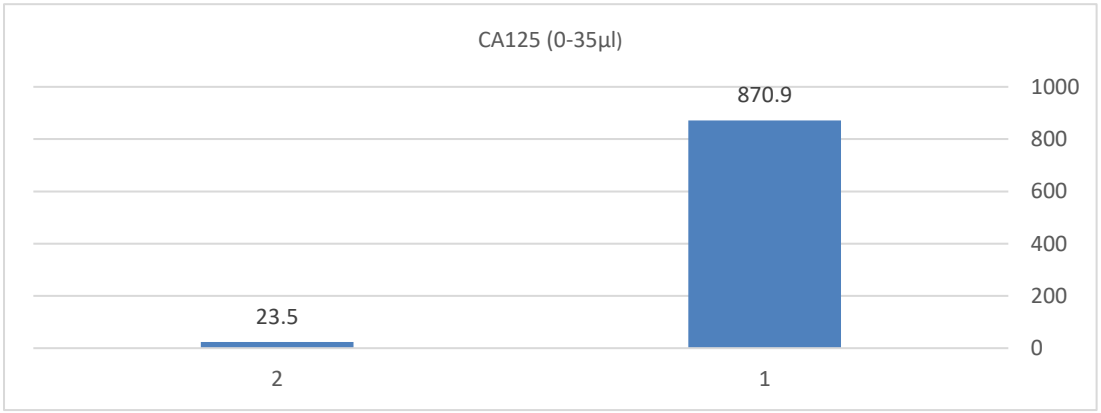
8- Stop the enzymatic reaction by adding (100 µl) of stop solution to each well.

9- Then the absorbance (OD) of each well was determined at (450 ± 10) nm with a micro-titter plate reader it is recommended that the wells be read within (10 minutes) after adding the stop solution.

3-



in ovary and uterus appearance of an increase, and it was the increase remains of highest among serous cancers with values exceeding (500 µ/ml) the normal rang(0-35µ/ml) (figur3-1).



epithelial ovarian cancer.

Figure 3-1-b: A curve representing the value of CA125 in Endometrial carcinoma

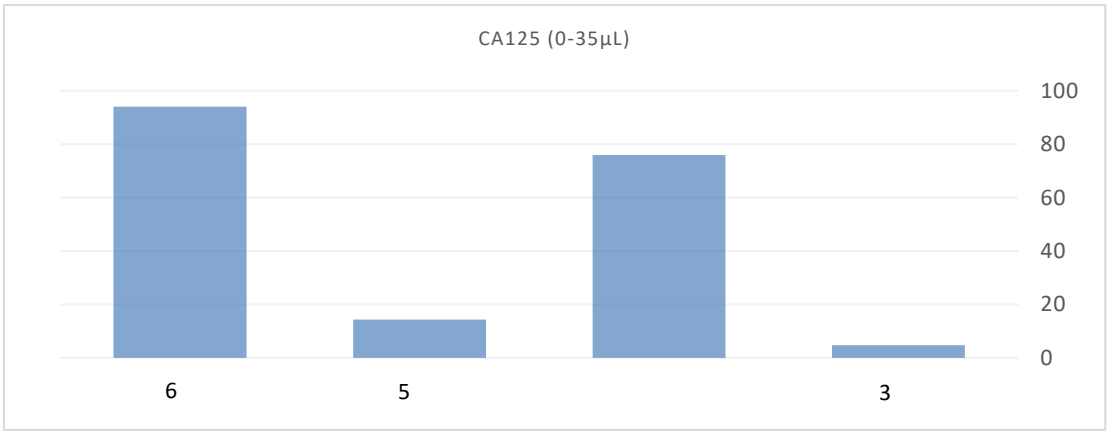


Figure 3-1-C: A curve showing the rise and fall of value of CA125 in Epithelial ovarian cancer and Endometrial carcinoma.

4- Discussion

4-1 CA125 tumor marker analysis

The prognosis for each subtype of ovarian cancer varies, making it a diverse illness. Patients with type I tumours often receive an early diagnosis and have a good long-term outlook (28). Patients with aggressive type II tumours, on the other hand, typically have a dismal prognosis for survival and are detected at advanced stages (29). The primary ovarian cancer biomarker for over four decades has been the high molecular weight glycoprotein CA125 (18).

The conventional tumor marker CA125 has long been used in the diagnosis of HGSC. It is elevated in 75.6% of serous carcinoma cases but in only 57.6% of Ovarian clear cell carcinoma (*OCCC*) cases, thus, CA125 is a poor marker for OCCC, with only a mildly elevated baseline value and a frequent incidence of false-negative results, however, CA-125 levels can be used for predicting advanced stage disease, suboptimal debulking and platinum-resistance with cut-off values of ≥ 46.5 U/mL, ≥11.45 U/mL, and ≥66.4 U/mL. Increased CA125 levels after the end of chemotherapy is significantly associated with shorter PFS and OS, so it also can be used as a valid indicator of the prognosis and efficacy of chemotherapy in patients with OCCC. Because there is currently no appropriate biomarker for OCCC, novel diagnostic markers are urgently required to improve early diagnosis and therapeutic stratification of the disease to provide more favorable prognoses and survivability not change (30.31).

In our study the results showed that in patients with high-grade serous ovarian cancer (Group 1), calcium-125 levels were significantly higher ($p<0.01$) than in mucosal cancers (Group 2), which were below the normal limit. In this research, CA 125 does not increase in Grade 1 and 2 endometrial

cancer (Group 3), but it does increase in Grade 3 (Group 4). However, calcium-125 values decreased in Grade 1 endometrial cancer (Group 5) but increased in Group 6 endometrial cancer in the ovaries and uterus, with values exceeding (500 µm/ML) the normal range (0-35 µm/ML). Diagnosis of ovarian cancer Ovarian cancer patients. Especially those with serous carcinoma, which is the most common and severe kind, had elevated levels of CA125 (18). Assessing the levels of CA125 may aid in the diagnosis of ovarian cancer, confirmation of ovarian cancer may need the use of imaging techniques and biopsies in cases when levels are found to be elevated(32).

Understanding the differences between the subtypes of ovarian cancer is crucial for establishing practical biomarkers. The expression of CA 125 varies throughout the ovarian cancer subtypes. When compared with other subtypes, hCG-type ovarian tumors and endometriosis express CA 125 more often CA 125 values are higher in seromas and lower in mucosal ovarian malignancies, it is shown that the expression patterns of CA 125 differ across Type I and Type II tumors, and Type I ovarian cancer usually affects younger patients and is often detected in its early asymptomatic stages. In comparison, a number of investigations have shown that individuals with Type II tumors have significantly higher levels of calcium-125 than those with Type I tumors, and 90% of ovarian cancer deaths and 75% of ovarian cancers are caused by Type II tumors (33). In our study, we agree with the researchers in determining the values of calcium-125 in ovarian cancers according to (Figure 3-1).With regard to the high values of the Type II tumors, especially high-quality serology.

Research on Ovarian Cancer has shown that Hsp70 is often overexpressed in ovarian cancer tissues in comparison to normal ovarian tissues, the increase in Hsp70 expression might be a reaction to the adverse circumstances present in the tumour microenvironment, importance of Hsp70 as a tumor marker in patients with colorectal adenocarcinoma (34).

In ovarian cancer, similar to other types of cancer, the absence of p16 activity may result in unregulated cell growth and heightened cell replication. This absence can be caused by genetic mutations, deletions, or promoter hyper methylation, which diminishes the effectiveness of p16 in suppressing CDKs (35).

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