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ORIGINAL STUDY

Evaluation of Circulating hPG80, CA15-3, Estrogen and Progesterone as Biomarkers in Breast Cancer Patients

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ABSTRACT

It is very important to find reliable biomarkers for early detection and monitoring of treatment response because breast cancer is still one of the most common cancers in women around the world. The goal of this study was to find out how clinically important different biochemical markers are in Iraqi women with breast cancer. Some of these signs are progesterone (PG), oestrogen (E2), cancer antigen 15-3 (CA15-3), and circulating progastrin (hPG80). There were 100 women in this study, and they were all between the ages of 20 and 70. There were three groups of people who took part: a healthy control group (n = 25), a group of newly diagnosed breast cancer patients who weren't getting treatment (Group 1, n = 27), and a group of breast cancer patients who were getting chemotherapy and radiation (Group 2, n = 48). After taking blood, ELISA was used to measure the levels of hPG80, CA15-3, oestrogen, and progesterone in the serum. Statistical analysis was performed using GraphPad Prism version 9.3. A p-value of less than 0.05 was considered statistically significant. The participants were categorized into three groups: healthy controls, newly diagnosed untreated breast cancer patients, and patients undergoing chemotherapy and/or radiotherapy. Both treated and untreated breast cancer patients showed significantly elevated hPG80 levels compared to the control group ($p < 0.0001$), with no significant difference between the two patient groups. Serum CA15-3 levels were markedly higher in breast cancer patients, particularly in untreated individuals. Estrogen levels were substantially reduced during chemotherapy and radiotherapy, indicating suppression of ovarian function, whereas newly diagnosed patients exhibited significantly higher estrogen levels. Progesterone levels were elevated in untreated patients compared to controls, but significantly decreased in treated patients. These results show that CA15-3 is still useful for tracking the progress of a disease and the effectiveness of treatment. hPG80, on the other hand, may be a good biomarker for diagnosing breast cancer. Changes in oestrogen and progesterone levels are another way to see how different treatment methods and hormonal pathways change the biology of breast cancer. This study highlights the potential of circulating hPG80 as a novel diagnostic biomarker in breast cancer, alongside the established role of CA15-3 in disease monitoring, and provides new insights into hormone profile changes in Iraqi patients.

Keywords: Estrogen, Progesterone, HPG80, Ca15.3, Breast cancer

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1. Introduction

Cancer is characterized by uncontrolled cell proliferation leading to tumor formation. Tumors may be malignant, with the ability to invade and metastasize to other tissues, or benign, remaining localized and exhibiting slow growth [1–4]. Breast cancer is one of the most common malignant tumours in the world, with more than two million new cases each year [5, 6]. Several risk factors increase the likelihood of its development, including alcohol consumption, physical inactivity, and obesity. Early detection remains essential for improving clinical outcomes [7–9]. Patients may present with manifestations such as tissue heterogeneity, nipple irregularities, or unaccountable weight loss [8, 10]. How breast cancer is treated depends on whether it is aggressive and spreading to nearby tissues or not, and whether it is non-invasive and only affects ducts or lobules [11] (Fig. 1).

The relatively long doubling time of cancer cells (approximately 100–300 days) provides a valuable window for early detection and timely intervention. Breast cancer (BC) is commonly classified according to tumor size, lymph node involvement, and the presence of distant metastases. In stage 0, the tumor remains confined to its site of origin. Stage 1 is characterized by limited invasion into surrounding breast tissue. In stage 2, the tumor increases in size and may extend further within the breast. Stage 3 involves spread to regional lymph nodes and the chest wall. Stage 4 (metastatic stage) is defined by the dissemination of cancer cells to distant organs, including the brain, liver, lungs, and bones [13, 14].

To treat BC and reduce the risks linked to the condition, early diagnosis, adjuvant chemotherapy, hormone therapy, and radiation therapy are essential [15]. In breast cancer (BC), both progesterone (PR) and oestrogen (ER) receptors are considered prognostic factors. In the early 1900s, radical mastectomy was used, but it wasn't as effective as other, less extreme surgical methods. Anti-HER2 targeted therapy, endocrine therapy, and chemotherapy have all been very effective at lowering the risk of BC and improving survival [16]. Because BC is a diverse illness, locoregional treatment may be adequate for small tumours, but further therapy is necessary for cancer that has spread to distant places. The main goals of treatment are surgical resection, sampling, axillary lymph node excision, and radiation therapy [17]. To better understand disease progression and treatment response, biomarker analysis has become an essential tool. Histological grade, obtained via core needle biopsy (CNB), axillary status, and tumor size are important parameters in BC. ER-alpha is a key predictive factor for advanced-stage patients responding to hormone therapy, which is non-toxic and suitable for long-term use [18]. Cancer antigen CA15.3 is elevated pre-surgically and serves as an important marker for evaluating response after chemotherapy [19]. CA15-3 is a common serum tumour marker for breast cancer in clinical practice. CA15-3 is a non-invasive, easy-to-get, and affordable tumour marker that can be used to quickly diagnose, monitor, and predict BC in its early, advanced, and metastatic stages [20–22]. Based on current knowledge, its clinical value within the normal range hasn't been tested yet. It was hypothesised that the recurrence of breast cancer (BC) would be influenced by elevated CA15-3, which were initially within normal ranges in patients with early BC. In healthy people, progastrin is made

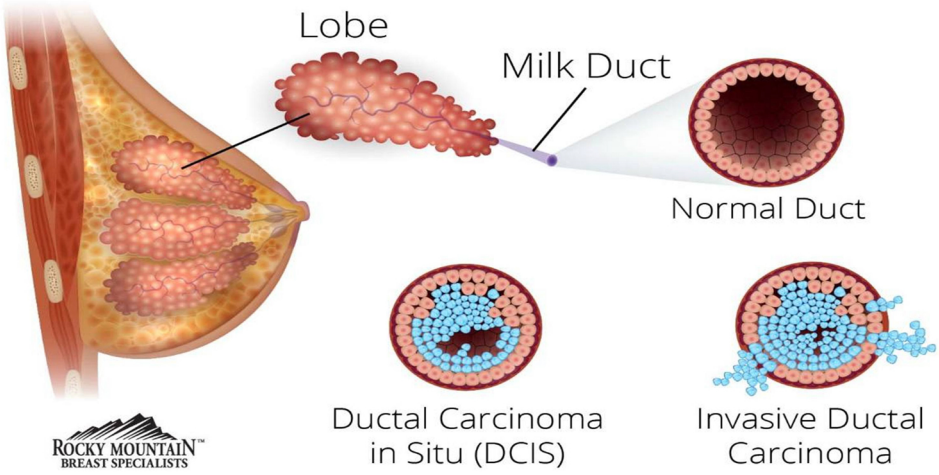

Fig. 1. Schematic illustration showing the difference between a normal milk duct, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma. In DCIS, abnormal cells proliferate within the duct without breaching the basement membrane, whereas in invasive carcinoma the cancer cells penetrate the duct wall and invade surrounding breast tissue [12].

by G cells in the stomach. It is the first step in making gastrin. The WNT/β-catenin oncogenic pathway, activated in numerous malignancies, directly regulates the GAST gene in tumour cells, responsible for encoding hPG80 [23, 24]. In contrast to stomach G cells, tumour cells do not turn progastrin into mature gastrin. A straightforward and cost-effective ELISA method can quantify this secreted unprocessed variant, referred to as hPG80 [23]. Numerous studies [25–27] have demonstrated a strong correlation between GAST gene activation, WNT pathway activation, and hPG80 expression in adenoma and cancer cells. hPG80 plays a crucial role in tumour growth by regulating cancer stem cell activities [28, 29], promoting angiogenesis [30], and influencing apoptosis [31]. Retrospective studies indicate that elevated levels of hPG80 correlate with unfavourable survival outcomes in glioblastoma [34], breast cancer [29], hepatocellular carcinoma (HCC) [33], and metastatic renal cell carcinoma (mRCC) [32]. It has been consistently demonstrated that factors regulating cell-specific proliferation and differentiation are pivotal in oncogenesis and may serve as viable therapeutic targets in advanced malignancies. Prolactin (PRL), along with the ovarian hormones progesterone and oestrogen, regulates the cycles of breast development and differentiation that enable successful lactation and the nourishment of infants. Mechanistic definitions of PRL-initiated signals that regulate the development of alveolar cells at birth and facilitate their expansion during pregnancy have been established [(35–38) and references therein]. Drawing parallels with the recognised functions of the two primary hormones governing mammary development and function, oestrogen and progesterone, prolactin (PRL) has been implicated in significant roles within these physiological processes related to breast cancer [(39-48) and references therein]. Nevertheless, understanding its mechanisms and impacts on diverse clinical breast cancers to formulate preventive or therapeutic strategies has proven challenging. It is well established that pituitary lactotrophs control PRL expression during pregnancy and lactation reviewed in (49)], but their expression in non-pregnant states has received less scrutiny. Many things affect the secretion of PRL from the pituitary gland, and the levels in women who are not pregnant are very different (50–52). Alongside physiological stressors, antipsychotics that inhibit dopamine lead to hyperprolactinemia (53, 54), and estrogen-progestin menopausal hormone therapy (MHT) elevates circulating prolactin levels (52). Moreover, breast cancer cells (58–61) and non-lactotrophs, including those in the mammary gland (55–57), are capable of expressing PRL. PGE2 activates NR4A, which can cause PRL expression in fibroblasts, especially in places where cancer might spread (62). Additionally, hGH acts as a strong PRL receptor agonist, which is not the case for growth hormone (GH) in nonprimates (63, 64). Breast cancer cells can produce it locally, similar to PRL, and hGH and PRL receptors are capable of heterodimerization (65). Consequently, even in the absence of pregnancy, agonists from local and systemic circulating sources may interact with prolactin receptors (PRLR) in the breast. However, the diagnostic and prognostic value of hPG80 in breast cancer, particularly in combination with CA15-3 and hormonal biomarkers, remains unclear, especially in underrepresented populations such as Iraqi patients.

2. Materials and methods

2.1. Study design and setting

Patient samples were obtained from the Oncology Teaching Hospital in Baghdad, Iraq, while control samples were collected from healthy women with no prior history of cancer. Patients with secondary breast tumors originating from other primary sites, liver disorders, chronic conditions such as diabetes mellitus and hypertension, polycystic ovary syndrome, and lactating women were excluded from the study. “This study was approved by the Research Ethics Committee of the College of Science, Al-Nahrain University, Baghdad, Iraq. Written informed consent was obtained from all participants prior to sample collection.”

2.2. Participants and sample collection

There were 100 Iraqi women in this study, all between the ages of 20 and 70. There were 25 healthy women who acted as a control group and 75 women with breast cancer (BC) at different stages of the disease. Each person gave a 5 ml sample of peripheral blood. The patient group was divided into two groups based on estrogen receptor (ER) status. There were 32 patients with ER-negative (ER–) tumors, which meant that the tumors did not express the receptor, and 43 patients with ER-positive (ER+) tumors, which meant that the tumors did express the receptor.

2.3. Parameter analysis

Blood samples were collected under controlled conditions, and serum samples were used to quantify estrogen, progesterone, HPG80, and CA15-3 levels. Biochemical analyses were performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (ELK Biotechnology and FineTest, Fine Biotech Co., Ltd., China), following the manufacturers’ instructions. Absorbance was measured using

a microplate ELISA reader (Germany) at the appropriate wavelength to ensure the accuracy of the results. All biomarkers were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols.

2.4. Statistical analysis

The data were analyzed using GraphPad Prism 9.3 Statistics. One-way analysis of variance (ANOVA) was used to compare the levels of the parameters. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cutoff value and assess diagnostic accuracy by comparing healthy controls with untreated breast cancer (BC) patients. A p-value of < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Evaluation of plasma Hpg80

The mean ± SD plasma hPG80 level in group 1 was 200.7 ± 37.64, while the mean ± SD level in the control group was 102.4 ± 22.59. The results showed a big difference between group 1 and the control ($P < 0.0001$). This result is in line with earlier studies that found that BC women's tissues had more hPG80 than healthy women's tissues [66, 69].

The mean ± SD of plasma hPG80 for Group 2 was 205.9 ± 24.17, which is higher than the control group (P-value < 0.0001).

The rise in BC patients suggests that hPG80 is strongly linked to the presence of tumours. Biologically, this rise can be explained by the fact that malignant epithelial cells release hPG80, a circulating progastrin peptide that has been linked to immune evasion, angiogenesis, and tumour growth.

The rise in Hpg80 levels in people who aren't getting treatment shows that it is a product of tumours and not a side effect of treatment. This result supports the idea that having BC in sick patients is linked to higher levels of HPG80. There was no clear difference between the untreated and treated BC groups (P-value = 0.7157), which means that cancer treatment did not have a big effect on HPG80 levels. The pre- and post-treatment levels of hPG80 in breast cancer patients following chemotherapy, radiation, or a combination thereof have not been recorded. This stability, on the other hand, means that cytotoxic or radiotherapeutic treatments do not have a big effect on hPG80. It could also be because of a delayed biomarker response—hPG80 may not go down right away after treatment, unlike metabolic markers, and it may take longer follow-up to see measurable changes [68]—or residual tumour activity—hPG80 secretion is probably kept up by malignant clones that survive after cytotoxic therapy [70]. These results show that hPG80 is a better diagnostic biomarker than a measure of therapeutic response. This means that it may be more useful for early diagnosis and long-term prognosis than for quick therapeutic monitoring [70].

Elevated hPG80 concentrations have been detected even in early-stage breast cancer, including stage I. This early rise [67] shows how important hPG80 could be for finding BC early. Integrating hPG80 testing into standard diagnostic protocols could significantly facilitate timely intervention and enhance prognosis, as early diagnosis is crucial for treatment efficacy and improved patient outcomes. The data for the analysis is in Table 1 and Fig. 2 shows it.

3.2. Evaluation of CA15-3

The current study found that people with breast cancer had much higher levels of CA15-3 in their blood than the healthy control group. The average CA15-3 level in Group 1 patients with untreated breast cancer was 37.50 ± 18.35 U/mL, which was much higher than the average level in the control group, which was 21.02 ± 1.93 U/mL ($p = 0.0001$). This result is consistent with previous research that found that breast cancer patients often have higher CA15-3 levels because malignant breast epithelial cells make and shed more of the MUC-1 glycoprotein into the bloodstream [71, 72]

CA15-3 levels in breast cancer patients undergoing chemotherapy and radiotherapy (Group 2) were significantly higher than those in the control group (29.24 ± 11.36 U/mL; $p = 0.039$). CA15-3 is widely used as a biomarker for monitoring treatment

Table 1. Plasma hPG80 levels (pg/mL) in control, untreated, and treated breast cancer groups.

Groups	Mean ± SD	P-values
Controls group	102.4 ± 22.59	< 0.0001
New cases group (Group 1)	200.7 ± 37.64	
Control group	102.4 ± 22.59	< 0.0001
Patient with chemo- and radiotherapy (group 2)	205.9 ± 24.17	
New cases group (Group 1)	200.7 ± 37.64	0.7157
Patient with chemo- and radiotherapy (group 2)	205.9 ± 24.17	

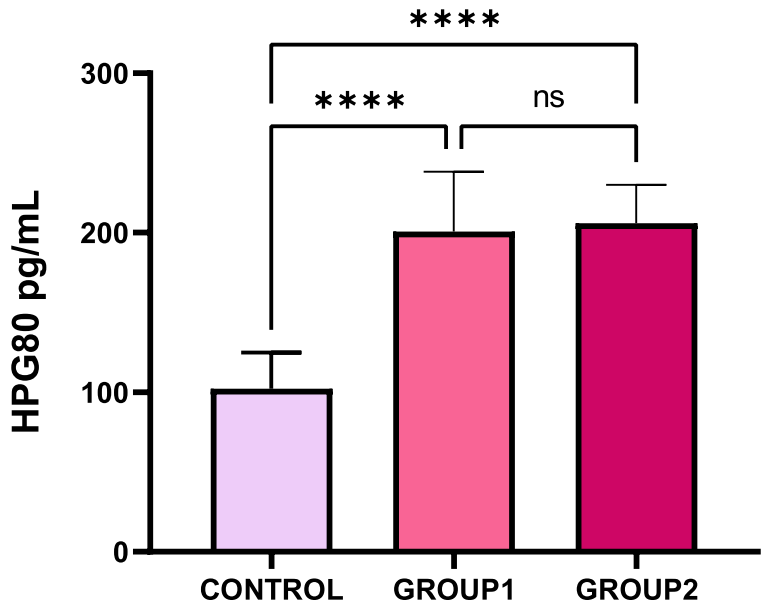

Fig. 2. Plasma hPG80 levels (pg/mL) in control subjects, untreated breast cancer patients (Group 1), and treated patients (Group 2).

response and disease progression in breast cancer. Elevated levels in treated patients may indicate residual tumor burden, ongoing tumor cell turnover, or suboptimal therapeutic response [73, 74]. A statistically significant difference was observed between untreated patients (Group 1) and those receiving treatment (Group 2) ($p = 0.0307$), with higher CA15-3 levels detected in untreated individuals. Previous studies have demonstrated that effective systemic therapy reduces circulating tumor markers, including CA15-3. Therefore, the observed reduction in CA15-3 levels following treatment may indicate a partial therapeutic response [75, 76] (Fig. 3).

In conclusion, our results show that CA15-3 is a useful biomarker for breast cancer patients to use for monitoring treatment and finding tumours (Table 2).

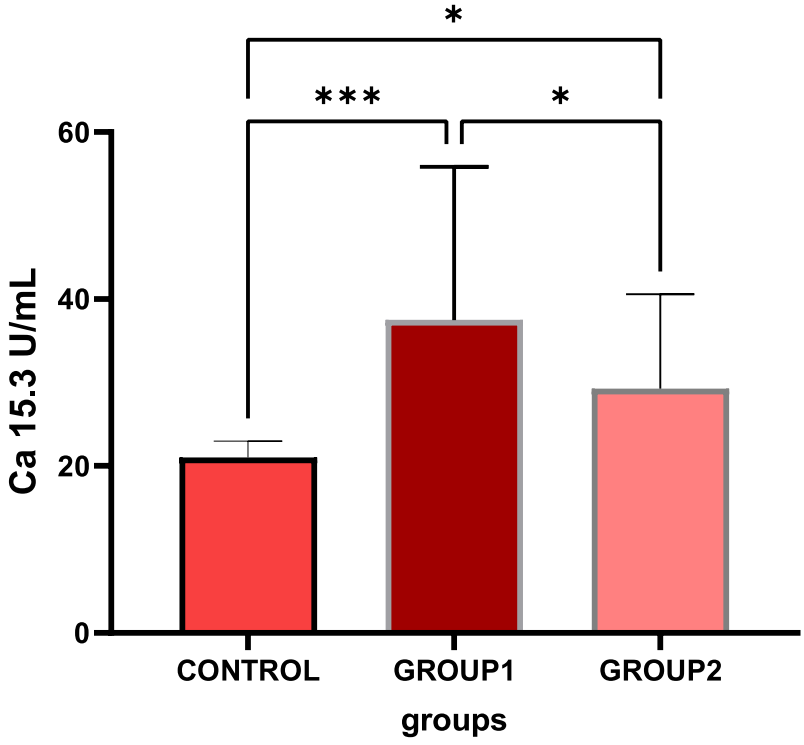

Fig. 3. Serum CA15-3 levels (U/mL) in control, untreated, and treated breast cancer groups.

Table 2. Serum CA15-3 concentrations (U/mL) among healthy controls, newly diagnosed untreated breast cancer patients (Group 1), and treated patients (Group 2).

Groups	Mean ± SD	P-values
Controls group	21.02 ± 1.933	0.0001
New cases group (Group 1)	37.5 ± 18.35	
Control group	21.02 ± 1.933	0.0390
Patient with chemo- and radiotherapy (group 2)	29.24 ± 11.36	
New cases group (Group 1)	37.5 ± 18.35	0.0307
Patient with chemo- and radiotherapy (group 2)	29.24 ± 11.36	

3.3. Evaluation of estrogen

The present study indicates that BC patients exhibited elevated mean serum E2 levels compared to healthy controls. The mean ± SD of E2 in the control group was 0.2014 ± 0.02418, but in patient groups 2 and 3, it was 0.8202 ± 0.3969 and 0.5606 ± 0.1659, respectively. There was a difference of $P < 0.00001$ between the patient and control groups. The increase can be attributed to the presence of ER+ patients, in whom circulating E2 stimulates tumour proliferation. On the other hand, Group 2, which got chemotherapy alone or with radiation, had lower E2 levels than Group 1 (P-value = 0.0001). The observed reduction in estrogen levels after chemotherapy is likely due to ovarian suppression and the gonadotoxic effects of chemotherapeutic agents, which impair normal ovarian function.

These results align with previous research indicating that the tissues of women with breast cancer exhibit elevated levels of E2 compared to those of healthy women, as well as the gonadotoxic effects of chemotherapy agents, which may lead to either transient or persistent ovarian insufficiency [77] effects on clinical practice. This results in two beneficial conclusions. First, circulating E2 accurately separates untreated BC patients from healthy controls in our dataset. This suggests that the disease is related to endocrine biology, especially in areas where ER positive is more common. Second, the big drop in E2 after chemotherapy shows how important it is to talk to patients about menopause symptoms and how to keep their fertility before they start taking cytotoxic drugs. When you check oestradiol levels along with ovarian reserve markers like AMH and FSH, you can objectively show that chemotherapy has caused ovarian suppression or failure because E2 levels in treated patients drop to or below control levels. Checking oestradiol levels may not be a direct tumour marker, but it gives us important information about how treatment affects ovarian function, which can affect fertility, menopausal symptoms, and long-term endocrine health. This pattern shows that oestradiol is likely to be a factor in the growth of hormone-dependent cancers and shows how much systemic therapy can affect ovarian function. Fig. 4 shows the analysis data from Table 3.

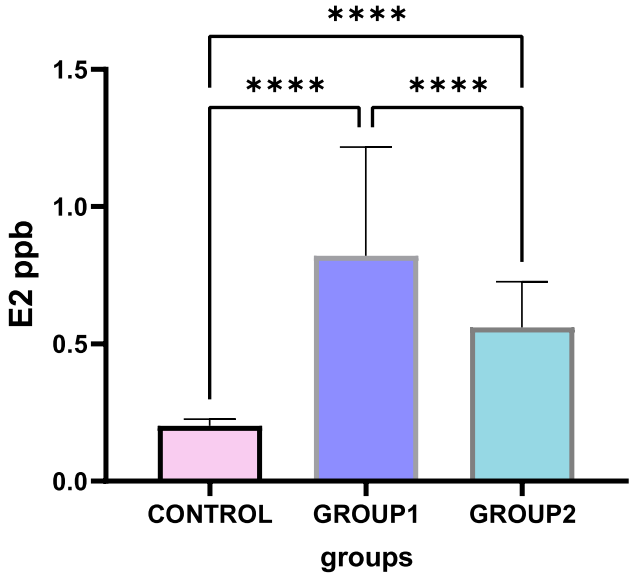

Fig. 4. Serum E2 levels (ppb) in control, untreated, and treated breast cancer groups.

Table 3. Serum E2 levels (ppb) in control, untreated, and treated breast cancer groups.

Groups	Mean ± SD	P-values
Controls group	0.2014 ± 0.02418	< 0.0001
New cases group (Group 1)	0.8202 ± 0.3969	
Control group	0.2014 ± 0.02418	< 0.0001
Patient with chemo- and radiotherapy (group 2)	0.5606 ± 0.1659	
New cases group (Group 1)	0.8202 ± 0.3969	< 0.0001
Patient with chemo- and radiotherapy (group 2)	0.5606 ± 0.1659	

3.4. Evaluation of progesterone

The present study indicates that BC patients exhibited elevated mean serum PG levels compared to healthy controls. Statistical analysis revealed significant group differences, highlighting PG's sensitivity to disease condition and treatment intervention. The mean ± SD serum PG level of 6.768 ± 1.603 ng/ml in the control group was within the normal range for women. The average level of newly diagnosed BC patients (Group 1) was 8.050 ± 2.063 ng/ml, which was significantly higher than the controls (P = 0.0125). In progesterone receptor (PR)-positive tumours, where progesterone signalling can modulate cell proliferation and influence tumour growth, this increase may indicate the involvement of ovarian steroid hormones in tumour biology. The relatively high standard deviation in Group 1 shows that there is a lot of variation between people. This is most likely due to receptor heterogeneity at diagnosis and during the menstrual cycle. Conversely, the combined therapy group (Group 3) exhibited a mean ± SD PG level of 5.759 ± 1.193 ng/ml, significantly lower than that of newly diagnosed patients (P < 0.0001) and controls (P = 0.0350). In general, the results show a clear trend: systemic therapy effectively stops progesterone, but it is much higher at diagnosis than in controls. This is probably because the ovaries are still working and the tumour biology is likely to be PR-positive.

This conclusion aligns with prior research indicating that the tissues of women with breast cancer exhibited elevated PG concentrations compared to those of healthy women, as well as the gonadotoxic effects of chemotherapy agents, which may lead to either transient or permanent ovarian insufficiency [78] (Fig. 5).

3.5. Diagnostic accuracy of HPG80, CA15-3, E2, and PG

The area under the curve (AUC) is a number that shows how well a test can tell the difference between healthy and sick people. Values close to 1.0 mean that the test is almost always correct [79]. Receiver operating characteristic (ROC) curve analysis remains a dependable and extensively utilized technique for evaluating the diagnostic efficacy of biomarkers.

While documented in certain biomarker studies, such flawless discrimination must be interpreted with caution, as it may be affected by sample size, population homogeneity, or overfitting effects [80, 81] (Table 4). E2 had an ideal diagnostic profile

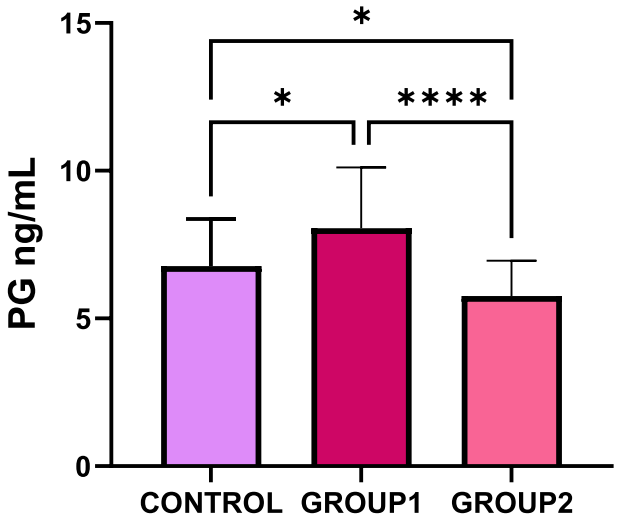

Fig. 5. Serum progesterone (PG) levels (ng/mL) in control, untreated, and treated breast cancer groups.

Table 4. Serum progesterone (PG) concentrations (ng/mL) among healthy controls, newly diagnosed untreated breast cancer patients (Group 1), and treated patients (Group 2).

Groups	Mean ± SD	P-values
Controls group	6.768 ± 1.603	0.0125
New cases group (Group 1)	8.050 ± 2.063	
Control group	6.768 ± 2.063	0.0350
Patient with chemo- and radiotherapy (group 2)	5.759 ± 1.193	
New cases group (Group 1)	8.050 ± 2.063	>0.0001
Patient with chemo- and radiotherapy (group 2)	5.759 ± 1.193	

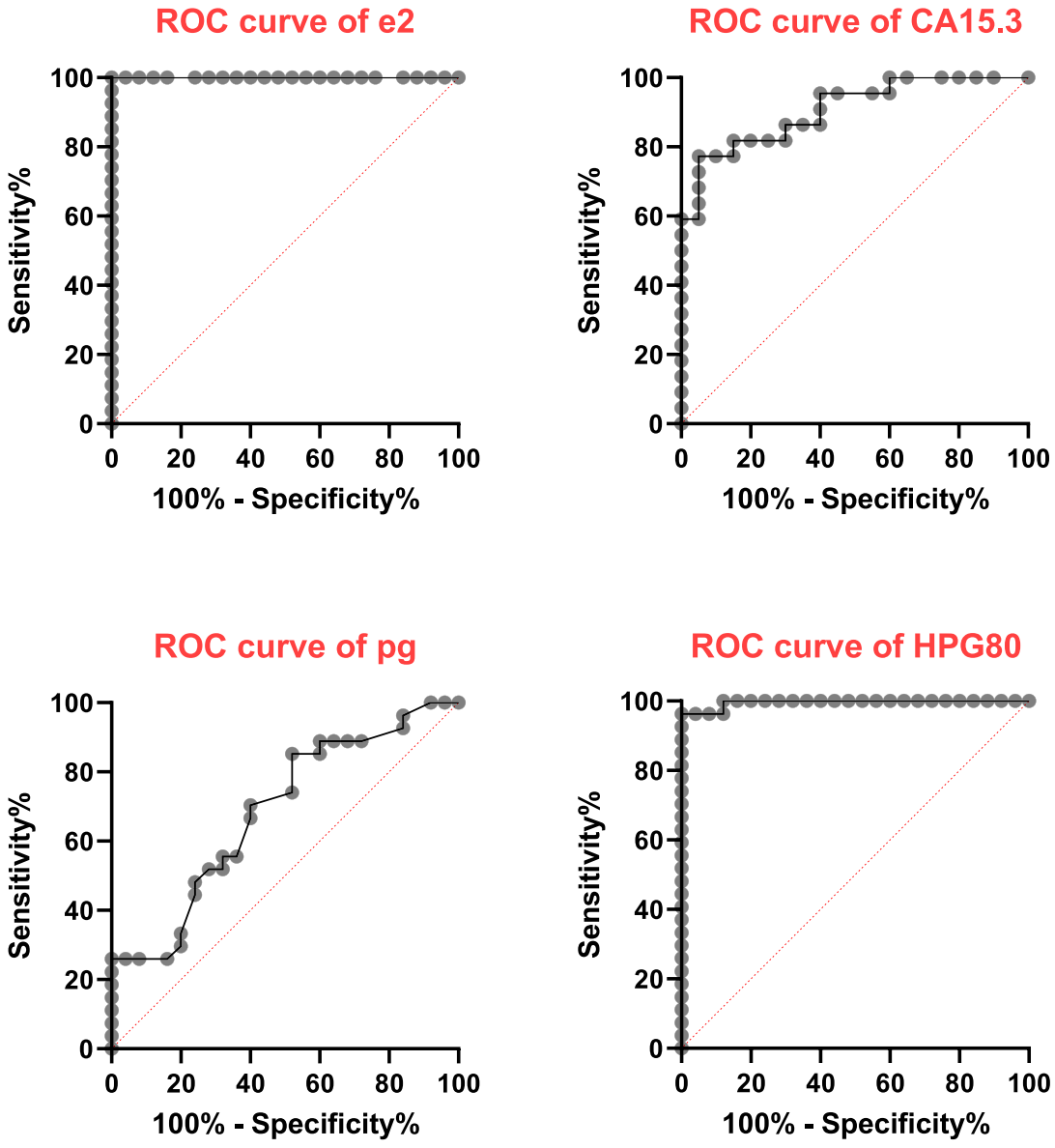

Fig. 6. Receiver operating characteristic (ROC) curves of estradiol (E2), CA15-3, hPG80, and progesterone (PG) for breast cancer diagnosis.

in this study (AUC = 1.000) with 100% sensitivity and specificity, which means that it could completely tell breast cancer patients apart from controls.

Hpg80 had an outstanding diagnostic accuracy (AUC = 0.9956). This makes it possible that it is a very sensitive molecular marker that is connected to biological processes that have to do with tumors, like how cells respond to stress and how tumors grow [79, 82] (Fig. 6].

The standard tumor marker CA15.3 had an excellent diagnostic performance (AUC = 0.9068), confirming its known role in diagnosing and keeping

Table 5. Receiver operating characteristic (ROC) analysis of hPG80, CA15-3, estradiol (E2), and progesterone (PG) for the diagnosis of breast cancer.

Untreated BC	AUC	Sensitivity%	Specificity%	95% CI	Cut-off	p-value
CA15.3	0.9068	77.27	95	0.8199 to 0.9938	> 24.33	<0.0001
Hpg80	0.9956	96.3	100	0.9847 to 1.000	> 149.4	<0.0001
E2	1.000	100	100	1.000 to 1.000	> 0.2710	<0.0001
PG	0.6844	85.19	48	0.5397 to 0.8292	> 6.150	0.0226

an eye on breast cancer. Earlier large-scale clinical studies [83, 84] found AUC values between 0.80 and 0.90 (Table 5).

The study found that CA15.3 is moderately sensitive (77.27%). This is in line with earlier research that showed that this marker is not very sensitive in early-stage breast cancer, even though it is more specific. This means that it is not very useful as a stand-alone screening tool [84, 85].

PG's diagnostic performance, on the other hand, was only "fair" because its AUC was so low (0.6844). This means that it isn't very selective and isn't very useful as a stand-alone diagnostic biomarker in the clinic [79].

It's important to remember that PG has a very high sensitivity (85.19%) but a very low specificity (48%). This means that a lot of the results are false positives. This can make diagnoses less accurate and make it seem like more people are sick than they really are in clinical settings [79, 86].

The very low p-values for E2, Hpg80, and CA15.3 ($p < 0.0001$) show that these biomarkers are strong and reliable. This is because statistical significance in ROC analysis shows actual discriminatory capability instead of random variation [83].

PG, on the other hand, is not as useful for diagnosis because it has a much lower statistical significance ($p = 0.0226$). This shows how important it is to use these markers along with more reliable biomarkers to make the diagnosis more accurate overall [79].

These findings underscore the capability of high-performing biomarkers to enhance early detection and diagnostic accuracy in breast cancer [79, 83]. Biomarkers with AUC values exceeding 0.9, such as E2 and Hpg80, are considered highly accurate diagnostic instruments, whereas markers with AUC values below 0.7, like PG, demonstrate restricted clinical utility.

4. Conclusion

The present study indicates that individuals with breast cancer exhibit significantly different levels of various circulating biomarkers compared to healthy individuals. Individuals, regardless of treatment status, demonstrated significantly elevated plasma hPG80 levels, indicating a strong correlation between the biomarker and tumour presence. This might be a better way to figure out what's wrong than to see how well treatment is working. People with breast cancer had very high levels of serum CA15-3, especially those who had just been diagnosed. This shows that the biomarker can be used in the clinic to keep an eye on the condition and see how well the treatment is working. Women who weren't getting treatment had more progesterone and oestrogen in their bodies. After chemotherapy and radiation therapy, their hormone and ovary levels went down because systemic treatment messed with them. In short, looking at hPG80, CA15-3, and hormonal biomarkers together gives us a lot of information about how breast cancer works and could help us keep an eye on patients and make more accurate diagnoses. To demonstrate the utility of these biomarkers in the detection, prediction, and assessment of breast cancer treatment, further research involving larger patient cohorts and extended follow-up periods is necessary.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

This study was conducted in accordance with established ethical standards and was approved by the Research Ethics Committee of the College of Science, Al-Nahrain University, Baghdad, Iraq (Approval No. 637, dated 3 November 2025). Written informed consent was obtained from all participants prior to sample collection, ensuring their voluntary participation and confidentiality of data.

Data availability

Not applicable.

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Authors' contributions

Safa Khaldoon collected the data from the literature and prepared the first draft of the manuscript. Khawla A. Kasar and Muna Bufaroosha reviewed the draft and contributed to its revision and improvement. Emad Yousif supervised the work, approved the final version of the manuscript, and served as the team leader.

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Code availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

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