How to Cite:
Hussein, A. A., Baban, R. S., & Mahdi, Q. A. (2022). Evaluation of aromatase as a potential biomarker and its association with CA 15-3 in patients with non-metastatic breast cancer. *International Journal of Health Sciences*, *6*(S5), 8339–8349. https://doi.org/10.53730/ijhs.v6nS5.10552

Evaluation of aromatase as a potential biomarker and its association with CA 15-3 in patients with non-metastatic breast cancer

Ammar Ahmed Hussein
Al-Nahrain University, College of Medicine, Department of Chemistry and Biochemistry, Baghdad, Iraq
Corresponding author email: a.biochem21@gmail.com

Rayah S. Baban
Al-Nahrain University, College of Medicine, Department of Chemistry and Biochemistry, Baghdad, Iraq

Qahtan A. Mahdi
M.B.Ch.B, F.I.C.S/Al-Nahrain University, College of Medicine, Baghdad, Iraq.

***Abstract*---**Breast cancer has been more common among Iraqi women in recent years. Monitoring proteins in human bodily fluids for early stage detection and prognosis of breast cancer is the best way and the first step toward effective therapy, delaying metastases and lowering mortality. In this study, we aimed to evaluate the value of Aromatase as a diagnostic and prognostic marker in breast cancer patients and to analyze the associations between Aromatase and standard CA 15-3 biomarker, as well as clinicopathological parameters. This study included 60 newly diagnosed patients, 49 receiving adjuvant hormone therapy, and 50 women who had a benign breast tumour as controls. We measured the serum levels by ELISA technique of Aromatase and CA 15-3. The results showed there were a significant difference in the mean serum Aromatase level amongst study groups ($p < 0.001$), and showed significant positive correlation with serum CA 15-3 in newly diagnosed group and treated group, ($P < 0.001$) and ($p = 0.041$), respectively. It also showed there were no significant correlations of serum Aromatase and CA 15-3 with menopause status, age, BMI, tumor size, node status, grade, tumor type, ER, PR and Her2. Serum ARO and CA-15-3 were excellent predictors by ROC, 93.3% and 88.3%, respectively, and the combination of the Aromatase ELISA and CA 15-3 biomarkers increased sensitivity to 96.7%.

***Keywords*---**Breast cancer, Aromatase, CA 15-3, Tumor.

Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

Introduction

Breast cancer (BC) is the most common type of malignant tumours detected in females. In 2020, it has surpassed lung cancer as the most commonly diagnosed malignancy, with an expected 2.3 million new cases, or roughly 11.7 percent of all malignancies in the worldwide (Sung *et al.*, 2021). This malignancy has a strong capacity for metastasis, resulting in a high fatality rate. Early detection improves its prognosis and increases its survival rate (Ragab *et al.*, 2018). Utilizing molecular biomarkers guarantees that patients with BC receive the appropriate treatment. Important roles are played by biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and HER2 in determining the prognosis and treatment for each individual patient (Tarighati *et al.*, 2022). Approximately 70% of breast cancer diagnoses are hormone receptor (HR)-positive, making them candidates for hormonal treatment (ET) such as tamoxifen and aromatase inhibitors (Robertson *et al.*, 2021).

Treatment decisions are crucial for patients with breast cancer; Aromatase (ARO) is one of the most hotly contested indicators. Cytochrome P450 aromatase (P450arom) is a microsomal enzyme, expressed in the endoplasmic reticulum, that catalyzes the final, rate-limiting step in estrogen biosynthesis, by converting 19-carbon steroids androgens (androstenedione and testosterone) into 18-carbon steroids estrogens, namely; estrone and estradiol (Bhardwaj *et al.,* 2019; Friesenhengst *et al.*, 2018). Aromatase is present in many tissues, including the gonads, brain, adipose tissues, placenta, blood vessels, skin, bone and in breast tissues (Bhardwaj *et al.,* 2019).

In the early studies, accurate estimations of aromatase expression and activity in breast cancer were difficult to use clinically due to unsatisfactory specificity and restricted availability of anti-aromatase antibodies (Santen *et al.*, 2009). Immunohistochemistry is a cost-effective method for the clinical assay. Currently, the breast cancer cases which may benefit of endocrine therapy are selected based on estimates of hormone receptors in resected tumor tissues, instead of directly assessing aromatase. The development of anti-aromatase antibodies with raised specificity and sensitivity made possible to best describe of aromatase immunoreactivity in tissues (Armasu *et al.*, 2020).

CA 15-3 is the MUC1 gene's protein product. It is a membrane associated glycoprotein that is mostly expressed on the apical side of glandular epithelial cells in alveoli and mammary ducts in normal mammary tissue. The MUC1 gene is usually highly overexpressed in the tissue of malignant breast cancers. Its expression has been shown to be more than 10 times higher in malignant breast carcinomas than in neighbouring normal glandular epithelium (Pedersen *et al.*, 2013; Coppola *et al.*, 2021).

CA 15-3 is a significant breast cancer biomarker that plays a critical role in breast cancer screening and has been widely adopted into clinical routine (Saadati *et al.*, 2019; Li *et al.*, 2020), However, the correlation between this indicator and breast cancer prognosis has not been conclusively established (Li *et al.*, 2020). The concentration of serum carcinoma antigen 15-3 gives valuable information for monitoring therapy, as well as for predicting the early diagnosis of breast cancer recurrence following curative surgery(Hasanzadeh *et al.*, 2018; Hassanpour *et al.*, 2019), especially in patients who cannot be evaluated using radiological methods (Saadati *et al.*, 2019). Since elevated levels of this tumour biomarker in serum tend to rise breast cancer severity or tumour size (Abed *et al.*, 2020).

In conjunction with imaging, disease history, and clinical course, an aberrant level or increase of CA-15.3 helps to diagnose tumour progression in metastatic breast cancer (Clatot *et al.*, 2020; Attia *et al.*, 2021), only, according to current the American Society of Clinical Oncology guidelines (Choudhury and Agarwala, 2018; Li *et al.*, 2020). In contrast, The European Group on Tumor Markers has suggested using CA15-3 and CEA to assess a patient's likely disease course, diagnose disease progression early, and select therapy options in breast cancer (Li *et al.*, 2020). Despite its relationship with BC cells, an increase in CA 15-3 can also be observed in benign illnesses such as liver disease and benign breast, lung, or ovarian disease; hence, it is not regarded a diagnostically specific biomarker for BC (Coppola *et al.*, 2021). Prior to now, there was insufficient evidence supporting the use of CA-15.3 instead of traditional follow-up.

The lack of predictive markers leaves the selection of administering endocrine therapy alone or in combination with other agent to empirical considerations. Given that a large part of HR+/HER2-neg patients will receive extended benefit from (ET) alone (Bonechi *et al.*, 2018), as well as, diagnoses and therapies are incomplete and many patients die due to recurrence; thus, improved diagnosis and prognosis using novel biomarkers is essential for improve the diagnosis and treatment of breast cancer (Ragab *et al.*, 2018). The aims of this study were to evaluate the values of serum Aromatase and CA15-3 as diagnostic and prognostic markers by ELISA in non-metastatic breast cancer patients to analyze the associations between ARO and other biomarkers in breast cancer patients, and to compare ARO and CA 15-3 to histological grading.

Material and Methods

From 16 October 2019 to 30 June 2021, this case-control study was done on 159 female patients with breast tumors, both malignant and benign. Women patients were recruited Baghdad Medical City. The practical part was conducted in the Research Laboratories of the College of Medicine/Al-Nahrain University's Department of Chemistry and Biochemistry. These groups consist of the following; (a) **Sixty** newly diagnosed women patients with early stages breast cancer. (b) **Forty-nine** patients with early stages of breast cancer using endocrine therapy for the first time. (c) **Fifty** newly diagnosed women with benign breast tumors as control group. The selection criteria were as follows: (a) All patients were assessed by a consultant physician and diagnosed with early-stages, non-metastatic breast cancer. (b) Absence of metastatic or recurrent breast cancer, as well as no neo-adjuvant therapy has been used. (c) Absence of systemic diseases such as hypothalamic or pituitary gland abnormalities, diabetes and hypertension. Chronic inflammatory conditions, hepatic, renal, or cardiac failure. After explaining study objectives and obtaining written informed consent from all patients, baseline demographic and clinical data were collected from participants by interview and recorded using a study questionnaire. Histopathological reports included the patient's age, tumor type, size, grade, nodal status, oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (Her2neu) status. The study was approved by the Institutional Review Board of the College of Medicine at Al-Nahrain University (approval date: 10/14/2019 and approval number: 5/1/52/1571).

Statistical Analysis

Statistical analysis was performed using SPSS program (version 22). Because all continuous variables were normally distributed, data were presented as mean ± standard deviation (mean±SD) and all statistical comparisons were done using the independent t-test, with a P value of 0.01 regarded statistically significant. Spearman rank correlation was used to analyze the relationship between the variables. Analyses were performed as a two-tailed, and the descriptive level of significance was set at $p < 0.001$. The Receiver Operating Characteristic "ROC" curve technique was used to establish the utility of any parameter as a diagnostic or screening tool for disease, as well as the capacity to calculate the "cut-off value" with the highest sensitivity and specificity for disease diagnosis.

Results

159 patients were examined during the study period. The patients under study were divided into three groups; 60 newly diagnosed women with breast carcinoma, 49 women with breast carcinoma receiving adjuvant hormonal treatment, and 50 women with benign tumors as control (Table 1.1)

Table 1.1: Comparison of mean age and frequency distribution according to age among study groups

Characteristic	Control group *n* = 50	Newly diagnosed BC *n* = 60	Treated BC *n* = 49	*p*
Age (years)				
Mean ±SD	45.79 ±10.46	50.02 ±10.68	47.67 ±10.59	0.279 O NS
Range	22 -70	32 -75	27 -75	
22-29, *n* (%)	2 (4 %)	0 (0.0 %)	2 (4.1 %)	
30-39, *n* (%)	16 (32 %)	10 (16.7 %)	8 (16.3 %)	
40-49, *n* (%)	12 (24 %)	19 (31.7 %)	17 (34.7 %)	
50-59, *n* (%)	11 (22 %)	18 (30.0 %)	17 (34.7 %)	
60-69, *n* (%)	8 (16 %)	11 (18.3 %)	4 (8.2 %)	
70-75, *n* (%)	1 (2 %)	2 (3.3 %)	1 (2.0 %)	

n: *denotes the number of cases;* ***SD****: is the standard deviation;* ***BC:*** *denotes breast carcinoma;* ***O:*** *denotes one-way ANOVA;* ***NS:*** *denotes not significant at $p > 0.05$.*

The results showed there no statistically significant differences in mean age between study groups ($p = 0.279$). The mean age of control group was 45.79 ±10.46 years, that of newly diagnosed women with breast carcinoma was 50.02 ±10.68 years and that of women with breast carcinoma receiving hormonal treatment was 47.67 ±10.59 years. The frequency distribution according to age showed that the majority of women with breast cancer were between 40 to 49 and 50 to 59 years.

The frequency distribution of breast cancer patients and control women by menopausal status showed that the control group included 28 (56 %) pre-menopausal women and 22 (44 %) post-menopausal women, the group of newly diagnosed breast cancer included 32 (53.3 %) pre-menopausal women and 28 (46.7 %) post-menopausal women, and the group of treated breast carcinoma included 29 (59.2 %) pre-menopausal women and 20 (40.8 %) post-menopausal women. There was no statistically significant difference in the frequency distribution of women with breast cancer and controls according to menopausal status (p = 0.698).

Evaluation of serum markers levels among study groups

The serum levels of Aromatase and CA-15-3 contrasted among study groups are summarised in Table 2. There was a significant difference in the mean ARO level amongst study groups ($p < 0.001$); the level was highest in newly diagnosed group followed by treated group and then by control group. There was also significant difference in mean CA-15-3 level among study groups ($p < 0.001$); the level was highest in newly diagnosed group followed by treated group and control group.

Table 2: The serum levels of Aromatase and CA-15-3 contrasted among study groups

	Control group *n* = 50	Newly diagnosed BC *n* = 60	Treated BC *n* = 49	*p*
Aromatase (ng/ml)				
Mean ±SD	3.81 ±0.60 B	8.39 ±2.86 A	3.98 ±0.62 B	< 0.001 O **
Range	3.02 -5.53	3.71 -16.81	3.02 -5.14	
CA 15-3 (U/ml)				
Mean ±SD	10.17 ±3.29 B	36.06 ±19.83 A	11.12 ±3.15 B	< 0.001 O **
Range	5.83 -17.40	11.23 -118.33	5.15 - 18.98	

n: denotes the number of cases; **BC**: breast carcinoma; **SD**: denotes standard deviation; **O**: denotes one-way ANOVA; **NS**: denotes not significant at $p > 0.05$; **: denotes significant at $p \le 0.01$; capital letters A, B, and C were used to express level of significance according to the post hoc LSD multiple comparison test; in such a way that different letters indicate significant difference while similar letters indicate no significant difference; and letter A takes the highest mean value followed by B and then C.

Correlation between serum biomarkers concentrations

Serum CA-15-3 showed significant positive correlation with serum Aromatase in in newly diagnosed group and treated group, < 0.001 and 0.041, respectively.

Correlations of serum markers with other parameters

Correlations of serum markers with other parameters in group of newly diagnosed breast cancer are shown in table 3. There were no significant correlations of serum ARO and CA 15-3 with menopause status, age, BMI, tumor size, node status, grade, tumor type, ER, PR and Her2.

Table 3: Correlations of serum markers with other parameters in group of newly diagnosed breast cancer

Characteristic	Aromatase		CA 15-3	
	r	*p*	*r*	*p*
menopause status	-0.153	0.242	0.162	0.215
age	-0.110	0.402	0.180	0.170
BMI	-0.093	0.480	0.090	0.492
tumor size	-0.051	0.696	0.211	0.106
node status	-0.168	0.199	-0.208	0.110
grade	-0.164	0.212	-0.080	0.546
type	0.122	0.351	0.175	0.182
ER	-0.165	0.207	0.029	0.825
PR	-0.077	0.558	0.076	0.563
Her2	-0.026	0.843	-0.121	0.358

The diagnostic potential of serum markers in detecting breast carcinoma

A receiver operator characteristics curve analysis was performed for serum markers and the results are displayed in table 4 and 5, and figure 1 through 3. Serum ARO and CA-15-3 were excellent predictors.

Table 4: Receiver operator characteristics (ROC) curve parameters of serum markers

Characteristic	Aromatase	CA-153
Cutoff	>4.36	>17.4
AUC	0.971	0.977
95 % CI	0.905 to 0.996	0.915 to 0.998
p	< 0.001**	< 0.001**
Sensitivity %	93.3	88.3
Specificity %	94.7	100.0
Accuracy %	97.1	97.7

AUC: denotes area under the curve; CI: denotes confidence interval; **NS**: denotes not significant at $p > 0.05$; **: denotes significant at $p \leq 0.01$

Table 5: Sensitivity and specificity of combined markers

Characteristic	Aromatase	Ca 15-3	Aromatase/CA15-3
Sensitivity %	93.3	88.3	96.7
Specificity %	94.7	100	94.7
Accuracy %	97.1	97.7	96.2

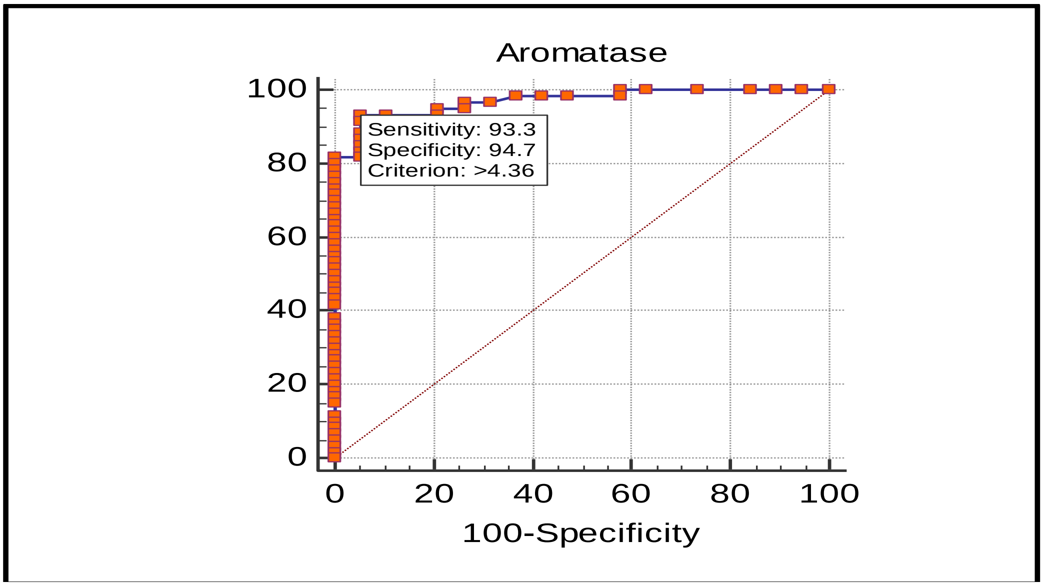

Figure 4.4: A receiver operator characteristic (ROC) curve to find the optimal ARO cutoff value for accurately diagnosing breast cancer

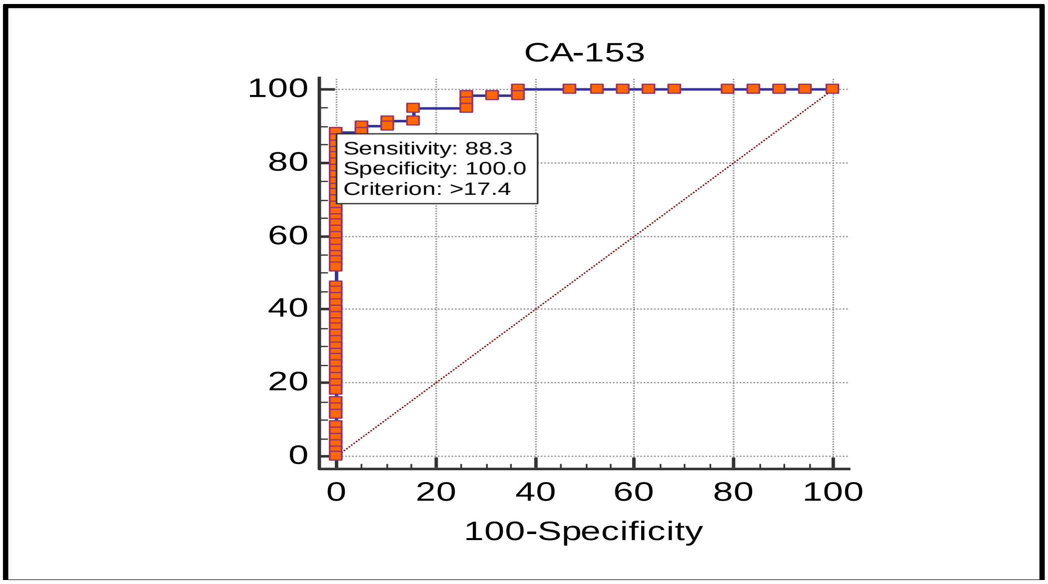

Figure 2: A receiver operator characteristic (ROC) curve to find the optimal CA-15.3 cutoff value for accurately diagnosing breast cancer

Discussion

Breast cancer is the most prevalent female cancer in the world and the second greatest cause of cancer-related death; therefore, it is essential to identify reliable prognostic markers that may identify patients at high risk of recurrence and select the most appropriate therapy for them (Ragab *et al.*, 2018). Since cancer is characterized by unrestricted cell expansion, cancer could be diagnosed using markers of cellular proliferation (Li, Wei and Song, 2018). Serum tumour biomarkers are soluble substances that are produced into the blood by tumour cells or in response to tumour cells by other cells. Serum tumour markers are the most often used in clinical settings due to their repeatability and ability to represent the dynamic progression of the illness. They are commonly used to predict recurrence or assess clinical outcome, to identify malignancies, and to monitor the response to anticancer therapy (Abed *et al.*, 2020).

The present study showed a significant difference in mean Aromatase level among study groups; the level was highest in newly diagnosed group followed by treated group and then by control group, with excellent predictors, when it carried out by receiver operating characteristics curve (ROC). Our result was in agreement with (Abd-Allateef, Hassan and Saleh, 2016) study, which reported that serum aromatase level significant elevated in post-menopausal patients with breast cancer in comparing with healthy group.

In contrast, other study done by (Hameed *et al.*, 2018) which found that aromatase activity level highly significant decrease in pre- and post-menopausal breast cancer patients compared to healthy control group. But did not illustrated the criteria of the study, whether the breast cancer cases were newly diagnosed or underwent surgery, chemotherapy or hormonal therapy. Thus may demonstrate of the incomprehensible results.

Estrogen is a unique product of the aromatase enzyme, is an serious risk factor for the development of estrogen receptor (ERα, encoded by ESR1 gene)-positive breast malignant (Zhao *et al.*, 2018; Reyes-Vázquez *et al.*, 2020). In pre-menopausal women, the production of estradiol (E2), a biologically active estrogen, occurs mainly in the ovaries, but aromatase expression and estrogen formation, mainly estrone (E1), also occur in the pre-adipocytes (fibroblasts) of breast adipose tissues and subcutaneous fat in other part of the body. When the ovaries cease of produce E2 in pos-tmenopausal women, local aromatase expression in the breast pre-adipocytes provides the major source of estrogens (both E1 and E2) that drive ERα positive breast cancer (Zhao *et al.*, 2018). Considering the low levels of circulating estrogen in post-menopause, it has been proposed that breast-derived estrogen is a crucial driver of breast cancer growth, and that decided tumors have the ability to further rise the locally production of estrogen. Consistent with this hypothesis, aromatase level and activity have been reported to be higher in quadrant of breast that containing the tumor (Brown *et al.*, 2017).

Also the present study showed there was no significant correlation between Aromatase level and Clinico-pathological characteristics; tumor size, nodal status, grade, ER/PR, HER2 status. In our study, number of patients was limited, and patients have been staged into I and II according to TNM system, therefore they are closely similar in their clinco-pathological characteristics, so no statistical significant differences were shown.

Several studies have been conducted to evaluate the clinical use of CA 15-3 as a breast cancer biomarker. CA 15-3 has a limited sensitivity for detecting early-stage tumors (Duffy, 2006), However, it is commonly employed for monitoring therapy in patients with advanced breast cancer (Park *et al.*, 2008). The sensitivity of the CA 15-3 test for breast cancer was up to 50% (Pedersen *et al.*, 2013). A combination of CA 15-3, CEA, and HER 2 enhanced sensitivity by 64% in metastatic patients (Lumachi F., 2004). In the present investigation, we observed strong correlations between serum Aromatase level and CA 15-3 values, and the combination of ARO and CA 15-3 biomarkers increased sensitivity from 93.3% and 88.3%, respectively, to 96.7%. The results of this study indicate that a combination of ARO ELISA and CA 15-3 will be particularly beneficial, as the two tests appear to provide complementary information.

According to our knowledge, this is the first large-scale study to demonstrate the diagnostic and prognostic value of Aromatase level in breast cancer. Clinico-pathological investigations of aromatase have been limited and difficult to use clinically due to unsatisfactory specificity and restricted availability of anti-aromatase antibodies (Santen *et al.*, 2009; Kanomata *et al.*, 2017). Thus, this explains the lack of previous studies.

The results of this investigation strongly imply that a combination of Aromatase and CA 15-3 will be extremely useful, as they appear to provide complementing data. In large case-control clinical investigations, further clinical examination of serum Aromatase levels in breast cancer treatment is highly justifiable.

Sources of Support: None.

Ethical approval: Al-Nahrain University, College of Medicine, the committee approved the study (approval date: 10/14/2019 and approval number: 5/1/52/1571).

Acknowledgements: None.

References

Abd-Allateef, M. W., Hassan, F. A.-A. and Saleh, W. A. (2016) 'Study the Effect of Anastrozole on Estradiol and Cytochrome P450 (Aromatase Enzyme) in Postmenopausal Breast Cancer Patients', *Journal of Al-Nahrain University-Science, 19*(2), pp. 43–50. doi: 10.22401/jnus.19.2.06.

Abed, S. N. et al. (2020) 'Serum levels of cancer antigen 15.3 and estrogen in a samples of iraqi women with breast cancer treated with anastrazole', *International Journal of Pharmaceutical Research, 12*(August), pp. 1604–1608. doi: 10.31838/ijpr/2020.SP1.246.

Armasu, I. et al. (2020) 'Insights on aromatase immunohistochemistry: Variations between intrinsic molecular subtypes of breast cancers', *Acta Endocrinologica, 16*(1), pp. 22–29. doi: 10.4183/aeb.2020.22.

Attia, M. S. et al. (2021) 'Terbium bipyridyl complex as a photo probe for the determination of carbohydrate antigen CA15.3 in different breast cancer patient samples', *Egyptian Journal of Chemistry, 64*(7), pp. 3541–3546. doi: 10.21608/ejchem.2021.66294.3421.

Bonechi, M. et al. (2018) 'Plasma thymidine kinase-1 activity predicts outcome in patients with hormone receptor positive and HER2 negative metastatic breast cancer treated with endocrine therapy', *Oncotarget, 9*(23), pp. 16389–16399. doi: 10.18632/oncotarget.24700.

Brown, K. A. et al. (2017) 'Menopause is a determinant of breast aromatase expression and its associations with BMI, inflammation, and systemic markers', *Journal of Clinical Endocrinology and Metabolism, 102*(5), pp. 1692–1701. doi: 10.1210/jc.2016-3606.

Choudhury, C. R. and Agarwala, N. (2018) 'A Study of Correlation of Pre-operative Serum CA-15.3 with Respect to Prognostic Factors and Early Recurrence in Breast Carcinoma in A Tertiary Care Centre in India', *International Journal of Contemporary Medical Research [IJCMR], 5*(10). doi: 10.21276/ijcmr.2018.5.10.12.

Clatot, F. et al. (2020) 'Risk of early progression according to circulating ESR1 mutation, CA-15.3 and cfDNA increases under first-line anti-aromatase treatment in metastatic breast cancer', *Breast Cancer Research, 22*(1), pp. 1–12. doi: 10.1186/s13058-020-01290-x.

Coppola, L. et al. (2021) 'The impact of different preanalytical methods related to CA 15-3 determination in frozen human blood samples: a systematic review', *Systematic Reviews, 10*(1), pp. 1–11. doi: 10.1186/s13643-021-01631-7.

Duffy, M. J. (2006) 'Serum tumor markers in breast cancer: Are they of clinical value?', *Clinical Chemistry, 52*(3), pp. 345–351. doi: 10.1373/clinchem.2005.059832.

Friesenhengst, A. et al. (2018) 'Elevated Aromatase (CYP19A1) Expression Is Associated with a Poor Survival of Patients with Estrogen Receptor Positive Breast Cancer', *Hormones and Cancer, 9*(2), pp. 128–138. doi: 10.1007/s12672-017-0317-2.

Hameed, E. H., Hussein, S. Z., & Abed, N. A. (2018) 'Evaluation Of Aromatase Activity In Pre And Postmenopausal', *WJPMR, 4*(9), pp. 99–100.

Hasanzadeh, M. et al. (2018) 'Ultrasensitive immunoassay of tumor protein CA 15.3 in MCF-7 breast cancer cell lysates and unprocessed human plasma using gold nanoparticles doped on the structure of mesoporous silica', *International Journal of Biological Macromolecules, 120*, pp. 2493–2508. doi: 10.1016/j.ijbiomac.2018.09.020.

Hassanpour, S. et al. (2019) 'A novel paper based immunoassay of breast cancer specific carbohydrate (CA 15.3) using silver nanoparticles-reduced graphene oxide nano-ink technology: A new platform to construction of microfluidic paper-based analytical devices (μPADs) towards biomedical analysis', *Microchemical Journal, 146*(January), pp. 345–358. doi: 10.1016/j.microc.2019.01.018.

Kanomata, N. et al. (2017) 'Preparation of a novel antiserum to aromatase with high affinity and specificity: Its clinicopathological significance on breast cancer tissue', PLoS ONE, 12(5), p. 183202. doi: 10.1371/journal.pone.0177439.

Li, J. et al. (2020) 'Tumor markers CA15-3, CA125, CEA and breast cancer survival by molecular subtype: a cohort study', *Breast Cancer, 27*(4), pp. 621–630. doi: 10.1007/s12282-020-01058-3.

Li, Y., Wei, R. and Song, S. (2018) 'Diagnostic and Prognostic Value of Serum Thymidine Kinase 1 in Cancer Patients', *Indian Journal of Hematology and Blood Transfusion, 34*(1), pp. 168–170. doi: 10.1007/s12288-017-0812-1.

Park, B. W. et al. (2008) 'Preoperative CA 15-3 and CEA serum levels as predictor for breast cancer outcomes', Annals of Oncology, 19(4), pp. 675–681. doi: 10.1093/annonc/mdm538.

Pedersen, A. C. et al. (2013) 'Sensitivity of CA 15-3, CEA and serum HER2 in the early detection of recurrence of breast cancer', *Clinical Chemistry and Laboratory Medicine, 51*(7), pp. 1511–1519. doi: 10.1515/cclm-2012-0488.

Ragab, H. M. et al. (2018) 'Assessment of Ki-67 as a potential biomarker in patients with breast cancer', *Journal of Genetic Engineering and Biotechnology, 16*(2), pp. 479–484. doi: 10.1016/j.jgeb.2018.03.002.

Reyes-Vázquez, L., Hernández, A. J. A. and Calderón-Aranda, E. S. (2020) 'Role of aromatase activation on sodium arsenite-induced proliferation, migration, and invasion of MDA-MB-231 and MDA-MB-453 breast cancer cell lines', Toxicology, 437(September 2019), p. 152440. doi: 10.1016/j.tox.2020.152440.

Robertson, J. F. R. et al. (2021) 'Meta-analyses of phase 3 randomised controlled trials of third generation aromatase inhibitors versus tamoxifen as first-line endocrine therapy in postmenopausal women with hormone receptor-positive advanced breast cancer', *European Journal of Cancer, 145*, pp. 19–28. doi: 10.1016/j.ejca.2020.11.038.

Saadati, A. et al. (2019) 'Immunosensing of breast cancer tumor protein CA 15-3 (carbohydrate antigen 15.3) using a novel nano-bioink: A new platform for screening of proteins in human biofluids by pen-on-paper technology', *International Journal of Biological Macromolecules, 132*, pp. 748–758. doi: 10.1016/j.ijbiomac.2019.03.170.

Santen, R. J. et al. (2009) 'History of aromatase: Saga of an important biological mediator and therapeutic target', *Endocrine Reviews, 30*(4), pp. 343–375. doi: 10.1210/er.2008-0016.

Sung, H. et al. (2021) 'Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', CA: A Cancer Journal for Clinicians, 71(3), pp. 209–249. doi: 10.3322/caac.21660.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Tarighati, E., Keivan, H., & Mahani, H. (2022). A review of prognostic and predictive biomarkers in breast cancer. *Clinical and Experimental Medicine*, 1-16.

Thaib, P. K. P., & Rahaju, A. S. (2022). Clinicopathological profile of clear cell renal cell carcinoma. *International Journal of Health & Medical Sciences, 5*(1), 91-100. https://doi.org/10.21744/ijhms.v5n1.1846

Zhao, H. et al. (2018) 'Adiposity Results in Metabolic and Inflammation Differences in Premenopausal and Postmenopausal Women Consistent with the Difference in Breast Cancer Risk', *Hormones and Cancer, 9*(4), pp. 229–239. doi: 10.1007/s12672-018-0329-6.