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NEW DIAGNOSTIC SERUM BIOMARKERS AND HORMON LEVELS IN WOMEN WITH BREAST CANCER

Leyla Karimova[a*], Gulnara Azizova[b], Ilaha Shahverdiyeva[b]

[a]*Department of Biophysics and Biochemistry, Faculty of Biology, Baku State University, 23 Z. Khalilov St, AZ1073, Baku, Azerbaijan**
[b]*Department of Biological Chemistry, Public Health Faculty, Azerbaijan Medical University, AZ1022, Anvar Gasymzadeh str., 14, Baku, Azerbaijan*

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Abstract

According to the World Health Organization, over the past decade, oncological diseases have remained a leading cause of morbidity and mortality world- wide. The article examines the role of serum matrix metalloproteinase-7 (MMP-7), MMP-9, and cysteine-rich angiogenic inducer 61 (CYR61) in the early diagnosis of breast cancer (BC). This research encompasses data from 74 women, aged 30-51, diagnosed with BC who visited the National Oncology Centre during 2023-2024. A control group consisted of 15 healthy women aged 25-38. Histochemical analysis determined the BC biotypes: 8 participants exhibited a "triple-negative" phenotype, 33 were Her2-positive, and 33 were Her2-negative. The control results were compared with both the collective data from all BC cases and specific subgroup indicators by biotype. The study found no statistically significant variation in biomarker levels across Her2-positive, Her2-negative, and triple-negative BC subgroups. However, significant differences in MMP-9 and CYR-61 levels were detected between the overall BC group and the control group ($p<0.001$). The study suggests that while serum biomarker levels do not differ significantly between BC biotypes based on Her2 secretion, the serum levels of MMP-9 and CYR-61 could be utilized as essential laboratory biomarkers for BC diagnosis irrespective of biotype.

Keywords: *breast cancer; matrix metalloproteinases; CYR-61; biomarkers; triple- negative; Her2-positive breast cancer*

1. Introduction

According to the World Health Organization, over the past decade, oncological diseases have remained a leading cause of morbidity and mortality worldwide. Statistical data indicate that breast cancer (BC) has surpassed other cancer types in growth dynamics in recent years, ranking second in cancer- related mortality among women globally. In Azerbaijan, BC remains the most prevalent malignancy among women, with an increasing incidence over the years. Data from the State Statistical Committee of the Republic of Azerbaijan indicate that in 2020, BC cases were recorded in 1,960 individuals, rising to 2,638 cases in 2021 and 2,541

[1] * Corresponding author. Tel.: +994 502365454
E-mail address: leylakarimovaaa@gmail.com

cases in 2023. When analyzing overall malignant neoplasm statistics by localization, BC ranked first, followed by lung cancer in second place and gastric cancer in third place. In 2023, lung cancer was diagnosed in 1,461 individuals, while gastric cancer was identified in 1,363 cases. That same year, the total number of newly registered cancer patients was 13,666, with the total number of recorded cancer patients in the country reaching 69,435. BC accounted for 18% of all malignancies in 2023, compared to 16.9% in 2020, 19.3% in 2021, and 18.4% in 2022.

Several risk factors contribute to the development of BC, including age, sex, obesity, harmful habits, exposure to radiation, early onset of reproductive processes, late first pregnancy, and genetic predisposition. Among these, age and sex are the most significant, with females over 40 years old constituting approximately 50% of BC cases. A family history of BC, particularly among male relatives, further elevates the risk. However, the absence of a family history does not eliminate the likelihood of developing the disease, as multiple environmental and genetic factors collectively contribute to its onset.

Despite widespread disease prevalence, advancements in early diagnostic techniques and treatment methodologies, BC-related metastasis and mortality rates remain high (Siegel et al., 2022). Developments in immunological and histochemical laboratory methods have enhanced diagnostic accuracy and prognosis evaluation. However, the increasing incidence of BC underscores the persistent significance of this public health challenge.In 2021, approximately 20% of diagnosed cancer cases in Azerbaijan were attributed to breast cancer, making it the second leading cause of death in the country [1-3].

Matrix metalloproteinases (MMPs), also known as matrixins, are proteolytic enzymes involved in extracellular matrix (ECM) remodeling. Recently, they have garnered interest as potential diagnostic markers for breast cancer. MMPs are integral to physiological processes, including embryonic develop- ment, morphogenesis, reproduction, and tissue regeneration. However, they are also implicated in pathological conditions such as arthritis, cancer, and car- diovascular diseases [4]. CYR61, a component of the ECM, participates in regu- lating cellular activities such as adhesion, migration, and apoptosis. The inves- tigation of ECM-related changes in breast cancer diagnostics may offer valuable insights for advancing diagnostic precision and prognostic accuracy [5, 6].

The study aimed to evaluate the diagnostic relevance of serum levels of MMP-7, MMP-9, and CYR-61 in women with breast cancer.

2. Materials and Methods

A prospective study was conducted with data collected from 74 women aged 30-51 diagnosed with BC who visited the National Oncology Centre in 2023-2024. Histochemical analysis identified BC biotypes: eight women exhibited the triple- negative phenotype, 33 were Her2-positive, and 33 were Her2-negative. A control group comprising 15 healthy women aged 25-38 was also analyzed.

MMP-7, MMP-9, and CYR-61 proteins and hormons levels were measured using enzyme-linked immunosorbent assay (ELISA). “Bio-Techne” (USA) reagent kits were used for analyzing proteins and “Diasino” (Spain) for all hormonal status levels, with results assessed on a STAT FAX 4700 microplate semi-automated immunoassay analyzer.

Statistical processing of the data employed both parametric and non-para- metric methods, with parametric processing via Fisher and t-Student Bonferroni criteria and non-parametric analysis using the Wilcoxon-Mann-Whitney test..

3. Results and discussion

In the breast cancer group, serum levels of estradiol, TSH, and prolactin hormones were higher compared to the control group. Prolactin levels in the breast cancer group ranged from 6.0 to 76.8 ng/ml, while in the control group, levels ranged from 8.9 to 14.1 ng/ml. Estradiol levels in the breast cancer group ranged from 1.8 to 122.1 ng/ml, whereas in the control group, levels ranged from 43.5 to 87.7 ng/ml. Progesterone levels were not significantly different in breast cancer patients. However, our results show that serum progesterone levels in breast cancer patients ranged from 0 to 1.3 ng/ml, whereas in the control group, the reference range was 0.1 to 0.4 ng/ml.

Estradiol, a potent estrogen hormone, has been extensively studied for its role in breast cancer development due to its mitogenic effects on breast tissue. Elevated serum prolactin levels have also been implicated in breast cancer, as prolactin promotes mammary gland development and may influence tumor

progression. Similarly, disruptions in thyroid function, as indicated by altered TSH levels, have been associated with changes in breast tissue homeostasis and cancer risk [7].

To further elucidate the relationship between these hormones and breast cancer, this study compares serum levels of estradiol, prolactin, and TSH between breast cancer patients and a healthy control group. Understanding these hormonal variations may provide insights into breast cancer etiology and highlight potential diagnostic or prognostic biomarkers.

In comparing Her2-positive, Her2-negative, and triple-negative BC groups to the control group, serum levels of MMP-7 were generally lower. Median values by subgroup were 1.2 ng/mL (range: 0.6-4.8) for Her2-positive,

1.2 ng/mL (range: 0.8-2.4) for Her2-negative, and 2.0 ng/mL (range: 1.1-4.9) for triple-negative. Although statistical significance was not observed in MMP- 7 levels between the primary and control groups according to Fisher's criterion (pf=0.464), a significant difference was detected using the Wilcoxon-Mann- Whitney test (pU<0.05). Healthy control subjects had an MMP-7 serum level of

3.1±0.7 ng/mL (Table 1).

Table 1. Serum levels of MMP7, MMP-9 and CYR-61 in different breast cancer subgroups and healthy control group members

Groups/ Parameters	MMP-7, ng/ml	MMP-9, ng/ml	CY-61, ng/ml R
Her2-positive, n=33	5,7 (0,2 – 32,2)	37,5 (8,0 – 140,0)	406,6 (0 – 5575,0)
Her2-negative, n=33	3,0 (0,2 –25,4)	73,1 (8,0 – 688,0)	277,5 (0 – 784,0)
Triple-negative, n=8	5,7 (0,4 – 29,2)	37,0 (12,0 – 72,0)	243,4 (37,0 – 683,0)
Total BC, n=74	4,5 (0,2 – 32,2)	53,3 (8,0 – 688,0) *	331,4 (0 – 5575,0)
Control group, n=15	3,1 (1,8 – 4,1)	486,9 (269,0 – 884,0)	13,3 (0 – 149,0)

MMP-9 levels varied from 28.4 to 46.5 ng/mL among Her2-positive par- ticipants, with an average of 37.5±4.4 ng/mL. In the triple-negative group, MMP-9 levels averaged 37.0±7.6 ng/mL. Comparisons among groups did not reveal significant statistical differences. However, the primary BC group's serum MMP-9 levels were significantly lower than those of the control group, demonstrating an 89.1% reduction (p<0.001). The difference remained statistically significant when analyzed with the t-Student-Bonferroni criterion (p<0.001).

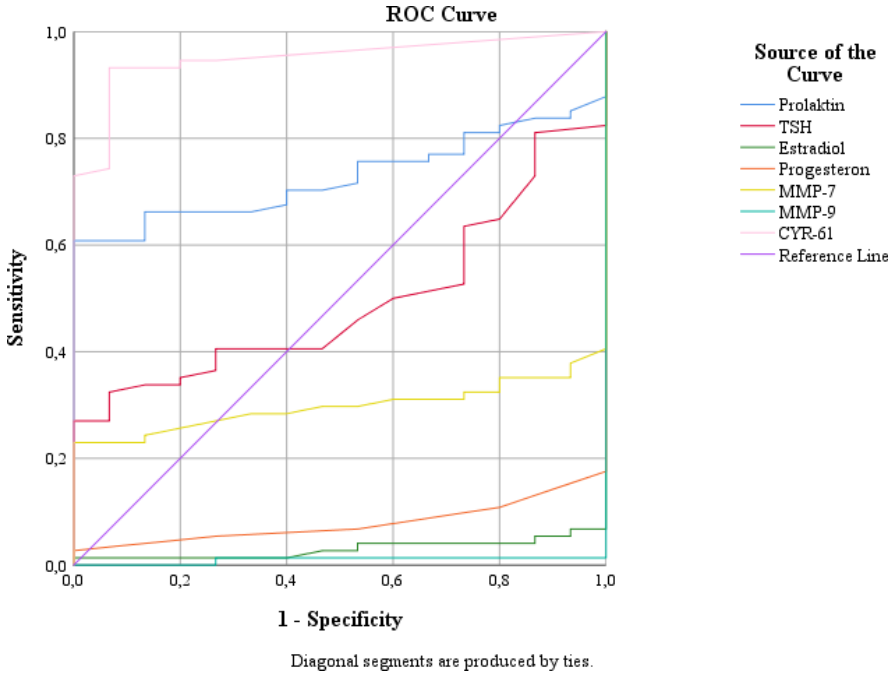
For CYR-61, no significant difference was observed between primary BC subgroups (pF=0.063), although the highest concentration was detected in the Her2-positive group, with levels 46.5% and 67.1% higher than Her2-negative and triple-negative groups, respectively. Serum levels of CYR-61 in Her2-positive, Her2-negative, and triple-negative groups were respectively 30.5, 20.8, and 18.3 times higher than those in the control group (pF<0.001).

The Receiver Operating Characteristic (ROC) curve is a fundamental statistical tool in medical research used to evaluate the diagnostic performance of tests or biomarkers. The ROC curve illustrates the trade-off between sensitivity (true positive rate) and specificity (false positive rate) across various threshold values of a diagnostic test. The area under the ROC curve (AUC) is a key indicator of a test's accuracy. AUC = 1.0 indicates a perfect test. AUC > 0.9 suggests excellent accuracy. AUC = 0.5 indicates a test with no diagnostic value (random guessing).

The ROC analysis helps assess the ability of biomarkers or clinical tests to distinguish between disease and non-disease states. It is widely used in studies involving cancer diagnostics, predictive models, and risk stratification. By comparing AUC values, researchers can identify the most effective diagnostic tools for clinical use. Our research results show that serum levels of CYR-61 and prolactin are essential biomarkers in breast cancer (Fig. 1).

Recent studies have demonstrated the role of Cyr61 in BC progression, particularly in its exosomal secretion regulated by c-Src activity [9]. Quantitative proteomic analyses of the secretome indicate that Cyr61 levels in the exosomal fraction are significantly reduced upon shRNA-mediated c-Src suppression, while intracellular Cyr61 expression remains unaffected [10, 11]. Cyr61 has been observed to colocalize with the cis-Golgi gp74 marker and the exosomal marker CD63, although c-Src depletion does not appear to alter their cellular distribution [12]. Further evidence from SUM159PT cells confirms that transient c-Src

inhibition leads to a decrease in secreted exosomal Cyr61 levels [13]. Additionally, the conditional expression of a dominant-negative c-Src mutant (SrcDN, c-Src-K295M/Y527F) in MDA-MB-231 and SUM159PT cells results in diminished Cyr61 secretion [14, 15]. Functionally, Cyr61 knockdown in MDA-MB-231 cells has been associated with a reduction in both invasive potential and transendothelial migration, suggesting its critical role in BC metastasis [16, 17].



Area Under the Curve					
Test Result Variable (s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Prolactine	0,727	0,050	0,006	0,629	0,826
TSH	0,495	0,064	0,948	0,370	0,619
Estradiole	0,030	0,017	0,000	0,000	0,064
Progesterone	0,079	0,029	0,000	0,023	0,135
MMP-7	0,298	0,051	0,014	0,197	0,398
MMP-9	0,010	0,010	0,000	0,000	0,030
CYR-61	0,950	0,024	0,000	0,903	0,997

Fig.1. ROC curves in total brast cancer group patients

4. Conclusions

In summary, although no statistically significant differences were iden- tified among the Her2-positive, Her2-negative, and triple-negative BC groups for MMP-7, MMP-9, and CYR-61 protein levels, a significant discrepancy in MMP-9 and CYR-61 levels was evident between the overall BC and control groups ($p<0.001$). The serum level of prolactin can also be used as a marker test in practice, together with other tests. These findings suggest that serum MMP- 9 and CYR-61 concentrations could be useful laboratory biomarkers in BC diagnosis, independent of biotype.

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