Research Article

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Are serum molecular markers more effective than the invasive methods used in the diagnosis of breast cancers?

Meme kanserlerinin tanısında, serum moleküler belirteçleri invazif yöntemlere göre daha mı etkilidirler?

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Abstract

Objectives: The most effective method currently used in breast cancer diagnosis is fine-needle aspiration biopsy. However, if the superiority of serum molecular markers to invasive diagnostic methods can be proven, it will have a great impact on the diagnostic approach and screening programs for breast cancer. The aim of this study is to demonstrate the efficacy of the serum molecular markers in the diagnosis of breast cancer with respect to invasive methods.

Materials and Methods: In this study, the literature on serum molecular markers and tissue molecular markers obtained using fine needle aspiration biopsy were collected. The absolute sensitivity rates obtained for serum molecular markers and for invasive needle biopsy methods were compared by systematic biostatistical analysis.

Results: In the diagnosis of breast cancer, the absolute sensitivity rates of serum molecular markers (90.6%) were found to be significantly higher than the absolute sensitivity rates of invasive methods (80.7%) ($p < 0.001$).

Conclusions: These results indicate that, serum molecular markers, can be used safely in breast cancer screening,

definitive diagnosis and follow-up. Therefore, in the near future, serum molecular markers are likely to take a higher priority in breast cancer diagnosis and screening.

Keywords: breast cancer; diagnosis; molecular markers.

ÖZ

Amaç: Günümüzde meme kanserinin tanısında kullanılan en etkili yöntem ince iğne aspirasyon biyopsisidir. Eğer, noninvaziv bir yöntem olarak, serum moleküler belirteçlerinin invaziv tanı yöntemlerine üstünlüğü kanıtlanabilirse, meme kanserinin tanı, tarama ve takibi programları üzerinde büyük etkisi olacaktır. Bu çalışmanın amacı, meme kanseri tanısında serum moleküler belirteçlerinin, invaziv yöntemlere göre daha etkin olduğunu göstermeye çalışmaktır.

Gereç ve Yöntem: Bu çalışmada, meme kanseri tanısında kullanılan serum moleküler belirteçleri ve ince iğne biyopsisi ile elde edilen moleküler belirteçleri içeren yayınlar derlenmiştir. Serum moleküler belirteçler yöntemi ve invaziv ince iğne biyopsisi yöntemi ile elde edilen mutlak duyarlılık oranları sistematik biyoistatistiksel analiz yapılarak değerlendirilmiştir.

Bulgular: Meme kanseri tanısında serum moleküler belirteçlerinin kullanıldığı çalışmalarda elde edilen mutlak duyarlılık oranları (%90.6), invaziv yöntemlerin kullanıldığı çalışmalarda elde edilenlere göre (%80.7) istatistiksel olarak anlamlı derecede yüksek bulunmuştur ($p < 0.001$).

Sonuç: Elde edilen sonuçlar, serumda bulunan moleküler belirteçlerin, meme kanseri taraması, kesin tanı ve takibinde güvenle kullanılabilecek noninvaziv bir yöntem olduğunu göstermektedir. Bu nedenle yakın gelecekte meme kanseri için tanısal yaklaşım ve tarama programlarında serum

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moleküler belirteçlerinin daha yüksek bir öncelik alması muhtemeldir.

Anahtar kelimeler: meme kanseri; moleküler belirteçler; tanı.

Introduction

Nowadays, in the diagnosis of breast cancer (BC), various non-invasive methods [1] (such as mammography, ultrasonography, doppler ultrasonography, elastography, magnetic resonance) and invasive methods [2] (serum molecular markers (MMs) and tissue molecular markers [3] obtained using fine needle aspiration biopsy (FNB) [4], core needle biopsy (CNB) [5], true-cut needle biopsy [6]) are used. Unfortunately, the noninvasive methods are ineffective in the diagnosis of small nodules. MMs have a key feature not only in the diagnosis but also in the prognosis and treatment of BC. When the molecular structure of a cancer cell is revealed by the analysis of MMs in BC cases, the way for personalized treatment could be provided.

In BC, there are two basic methods related to molecular diagnosis: MMs studied in tissues (or cell samples) and MMs studied in serum. In a study conducted by Radojicic et al., it was found that in triple negative breast cancers (TNBC), miR-21, miR-210 and miR-222 were significantly overexpressed, whereas miR-10b, miR-145, miR-205 and miR-122a significantly underexpressed [7]. Tang et al. [8] has shown that colorectal cancer associated transcript 1 (CCAT1) is a novel long noncoding RNA (LncRNA) plays an important role in the diagnosis and treatment of BC. Stergio et al. [9] demonstrated that mucin 1, a cell surface associated antigen (MUC1) is a much more important biomarker (BM) than the other known biomarkers such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in BC. In a study carried out by Menekşe et al. [10], anti MUC1 IgG levels in nipple aspirate fluid, in different BC subtypes, were investigated and were found significantly higher in TNBC, ER negative tumors, HER2 neu positive tumors and could be used to predict aggressiveness of BC.

In addition, in BC, vascular endothelial growth factor-C (VEGF-C) and Twist (T) [11], dual specific phosphatase 4 mRNA expression [12], TC or BC allele formation [13], Schlafen Family Member 11 (SLFN11) mRNA expression [14], phosphoinositide 3-kinase catalytic subunit (PIK3CA) expression [15], Phosphatidylinositol binding clathrin assembly protein (PICALM) gene interacting mitotic regulator (PIMREG) [16] have been reported to be highly effective as prognostic MMs.

Apart from these, in studies related to molecular treatment of BC: miR-125a-5p and miR-181a-5p [17]; indole-2-carboxamide derivative LG25 have anti TNBC potential, serine-threonine kinases Aurora A (AURKA), and p21-activated kinase 1 (PAK1) [18, 19]; miR-381 in [20] were reported in literature that they are very effective molecules in tumor suppression.

Discovery of new MMs related to the diagnosis of BC will have high potential in the effective diagnosis and treatment of BC. Therefore, in this study, it was aimed to investigate the effectiveness of serum MMs with respect to invasive FNB/CNB methods in the diagnosis of BC.

Materials and methods

Design of the study, inclusion and exclusion criteria

In this study, the recently published articles in literature about the MMs used in the diagnosis of BC were systematically reviewed. The articles reporting the values of MMs in serum and the values obtained by FNB/CNB methods in tissues were compared. The reason for including only FNB/CNB as invasive methods to this study is that, they are still the most effective methods used in BC diagnosis. Since it has the highest potential as a noninvasive definitive diagnostic method, serum MMs (although studies in serum is an invasive process, it is assumed as noninvasive by researchers) were also included. Studies performed with MMs in tissues for the diagnosis of BC were not included in this study because it is also an invasive method. In order to reveal the effects of MMs in serum, the data obtained from studies conducted with serum MMs and the data obtained from studies with FNB/CNB were compared. The number of the studies performed with MMs in serum is very limited. Therefore, all studies that are found in literature were included. In this study, only articles reporting histopathologically definitive diagnosis (gold standard), were included; in which, either noninvasive MMs and invasive FNB/CNB methods were used for the diagnosis of BC. When comparing diagnostic methods, absolute sensitivity (AS) rate (the ratio of cases diagnosed with BC by serum MMs or invasive methods, to the cases diagnosed with BC histopathologically) was taken as basis.

The BC cases in which the articles investigating the levels of MMs in serum were included; in a study by Heneghan et al. [21], miR-195 levels in serum in 83 BC and 44 control cases; in a study by Han et al. [22] combined miR-21, miR-155 and miR-365 levels in 99 BC and 21 control subjects; in a study by Shihomura et al. [23], in the serum of 1280 BC and 2,836 non-cancer control cases, miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, miR-6875-5p levels; in a study by Marwa et al. [24], histidine rich glycoprotein RNA levels in 60 BC and 30 control group cases were investigated.

Articles investigating absolute sensitivity rates using invasive FNB and CNB methods in the diagnosis of BC that are included in this study; FNB findings reported by Kazi et al. [25] in 698 BC patients; in a study by Ohashi et al. [26], FNB findings in 238 BC cases, CNB findings in 133 BC cases; in a study conducted by Kurita et al. [27], FNB findings in 182 BC cases, CNB findings in 56 BC cases, and combined FNB/CNB findings in 43 BC cases. In the study conducted in the A3 group, AS

results obtained by the researcher with the micro RNA panel according to the stages of BC: Stage 0, A3A; Stage 1, A3B; Stage 2, A3C; Stage 3, A3D; Stage 4, A3E; N0, A3F and M0, is numbered as A3G.

In BC cases diagnosed with FNB method; B2 and B3, the AS results of the same researcher (Ohashi et al.), FNB (B2) and CNB (B3) in BC cases; in Kurita's study: FNB (B4), CNB (B5), combined FNB/CNB methods are shown with (B6) [26, 27].

The studies included in this study is summarized in Table 1. The group in which MM analysis was performed in serum; named as group A (GpA), FNB and CNB applied group is named as group B (GpB) (Table 2). In this study, the recently published articles (2010–2020), in journals indexed in EMBASE and MEDLINE, reporting serum MMs and tissue FNB/CNB data, are included.

Statistical analysis

SPSS 23.0 package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and

Table 1: Data reported in literature for serum MMs and for invasive FNB and CNB methods in BC patients.

MMs in serum	No. of control	No. of BC	Ref. No.
miR-195	44	83	[15]
miR-21, miR-155, miR-365	99	21	[16]
miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, miR-6875-5p	2,836	1,280	[17]
His rich glycoprotein RNA	60	30	[18]
FNB and/or CNB in tissues			
FNB	–	698	[19]
FNB	–	238	[20]
CNB	–	133	[20]
FNB	–	182	[21]
CNB	–	56	[21]
Combined FNB and CNB	–	43	[21]

Table 2: Absolute sensitivities (%), mis- (MD) and correct diagnosis (CD) (%) in MM and FNB studies with BC patients.

Cancer diagnosis	MMs GpA (n=3,671)	FNB/CNB GpB (n=1,376)	Total	p-Value
BC (–)	125 (3.4)	176 (12.8)	301 (6.0)	p<0.001
BC (+)	3,546 (96.6)	1,200 (87.2)	4,746 (94.0)	
Absolute sensitivity	90.6	80.7	–	–

GpA, Group A cases; MMs, molecular markers; GpB, Group B cases; FNB, fine needle aspiration biopsy; CNB, core needle biopsy; BC (–), histopathologically positive but it is found as negative due to the limitation of the method. BC (+), both histopathologically and by the method it is found as positive. Values in parenthesis represent the percent of that group in the total number of patients in the same group.

percentages. In comparison of categorical variables, Chi-square test and Fisher's Accuracy Test were applied. Statistical level was taken as 0.05 in all tests.

Results

In this study, the absolute sensitivity rates in GpA (the results reported for serum molecular markers) and GpB (the results reported for invasive FNB and CNB) were compared. It was observed that the success rate in the studies conducted in GpA (90.6%) was statistically significantly higher than the studies in GpB (80.7%) ($p<0.001$) (Table 2). The percent absolute sensitivity rates (AS%) vs. mis-diagnosis (MD%) and correct diagnosis (CD%) in GpA cases and GpB cases are shown in (Table 2) (Figure 1).

When the results of the reported studies with MM and FNB (CNB), were compared, it was observed that the most effective diagnostic methods, in decreasing order, are the A3D, A3B and A3F (N0), A3A, A3G (M0), B4 and B3 methods (Table 3). The percent ASs vs. diagnosed and mis-diagnosed BC patients, at different stages of the disease determined using MM and FNB/CNB methods, is shown in Figure 2.

The most successful accurate ASs studies in GpA were carried out by Shihomura et al. [23], in their study, they performed five microRNA panel; A3D, A3B, A3F, A3A, A3G, (stages 3, stage 1, N0, stage 0 and M0 cases). The average absolute sensitivity, specificity and accuracy obtained by using five miRNA panel in serum, at stages 0, 1, 2, 3, 4, N0 and M0, were 97.3, 98, 98.1, 95.6, 100, 96.2, 98.1 and 97.3%, respectively (Figure 2) [23]. In the studies conducted with FNB and CNB methods (GpB), the ASs were found in a decreasing order that B4 (93.3%), B3 (91%), B1 (89.7%), B5

Figure 1: The percent AS vs. percent correctly diagnosed (%CD) and percent mis-diagnosed (%MD) BC patients plot, using MM and FNB (CNB) methods.

Table 3: Absolute sensitivities (%), mis- (MD) and correct diagnosis (CD) in MM and FNB studies with BC patients.

Author method Group code & Stage of BC	Cancer Diagnosis		AS, %	Total	Ref. No.
	Negative	Positive			
	BC (–)	BC (+)			
A1A; miR-195	12	71	85.5	83	[15]
A2; miR-21, miR155, miR365	15	84	84.8	99	[16]
A3A; stage 0	5	251	98.0	256	[17]
A3B; stage 1	9	474	98.1	483	[17]
A3C; five miRNA panel; stage 2	17	375	95.6	392	[17]
A3D; five miRNA panel; stage 3	0	22	100.0	22	[17]
A3E; five miRNA panel; stage 4	2	51	96.2	53	[17]
A3F; five miRNA panel; N0	20	1,050	98.1	1,070	[17]
A3G; five miRNA panel; M0	31	1,122	97.3	1,153	[17]
A4; HRG protein	8	52	86.6	60	[18]
B1; FNB	72	626	89.7	698	[19]
B2; FNB	59	179	75.2	238	[20]
B3; CNB	12	122	91.0	134	[20]
B4; FNB	13	182	93.3	195	[21]
B5; CNB	8	48	85.7	56	[21]
B6; combined FNB + CNB	12	43	78.2	55	[21]

GpA, serum molecular marker group; GpB, fine needle biopsy group; FNB, fine needle aspiration biopsy; CNB, core needle biopsy; BC, breast cancer; MD, misdiagnosis; BC (–), histopathologically positive but it is found as negative due to the limitation of the method; BC (+), both histopathologically and by the method it is found as positive.

Figure 2: The percent AS vs. correctly diagnosed (%CD) and misdiagnosed (%MD) BC patients plot, at different stages of the disease were determined using MM and FNB (CNB) methods.

(85.7%), B6 (78.2%) and B2 (75.2%) as given in Figure 2 and Table 3 [26, 27].

Discussion

MMs used in cancer diagnosis have several advantages. The two most important of these advantages are; 1. Even in very small tumors, their expression increases in blood or/and tissue. 2. Some of these markers are tumor-specific, thus providing tumor-specific information to the person. This information can be used not only in diagnosis, but also in prognosis and treatment.

Needle biopsies, especially in the last 10 years, are the most commonly used method for MMs studied in tissue samples. In addition, MM analyzes were performed in serum and in body fluids other than serum. The most important drawback of needle biopsies in BC is the implantation of tumor cells, few cases have been reported in the literature for BC [28, 29].

In BC patients, molecular analysis of cancer cells enables us to be effective in treatment as well. In a study conducted by Tan et al. [30], they performed that miR-491 targets TPX2 gene and plays a tumor suppressor role in BC. In a comparative study conducted by Alfarsi et al. [31], an increase in CDC20 miRNA expression in the ER positive subgroup of in BC patients has been shown, characterized by poor clinical outcome meaning poor response to endocrine therapy. In a study conducted by Cai et al. [32], transcriptional intermediary factor 1γ (Tif1γ) levels were measured in the serum of patients with BC to investigate the relationship between Tif1γ and overall survival (OS) and it was found that the Tif1γ positive patients have longer OS than the Tif1γ negative BC patients. Thus, the Tif1γ plasma levels are an independent prognostic factor and should be considered as a candidate to be an important prognostic MM in the BC [33].

The analysis of MMs in serum provides additional advantages. In a study by Oloomi et al. [33], the levels of some MMs (carcinoembryonic antigen, ERβ, cytokeratin19 and proto-oncogene) in tissue and serum in patients with BC were compared. Although, the difference between the tissue levels of these MMs, was not significantly different in healthy subjects and BC patients but their serum levels were significantly different [33]. Thus, this study indicates that some MMs detected in serum have superiority over tissue MMs in the diagnosis of BC [33].

The molecular analysis in cancer cells circulating in body fluids other than serum give promising results in the diagnosis of BC. When making molecular analysis

in body fluids, the major problem is the inability to ensure the homogeneity of the circulating cancer cells. Cheng and colleagues have developed the Hydro-Seq, a scalable hydrodynamic scRNA-seq barcoding technique, to obtain the homogeneity and catch cancer cells more easily, achieving successful results in their study on 21 patients [34].

In a study conducted by Wang et al. in 252 BC, 82 benign breast tumors, 127 healthy controls; preoperative and postoperative serum values of miR-21 were measured, expression levels were searched in patients with and without metastasis, and the effects of inhibition of miR-21 on BC metastasis and growth were investigated [35]. The result of their study indicates that serum levels of miR-21 were significantly higher in BC patients but decreased significantly after surgery [35]. On the other hand, higher expressions in patients with metastasis than patients without metastasis were observed and BC growth and metastasis decreased significantly with the inhibition of miR-21 expression [35].

According to the results obtained in this study, ASs obtained with serum MMs (GpA), appears to be significantly superior to the results obtained with the FNB and CNB (GpB) method in BC (Table 2; Figure 1). In addition, when the results in the published studies included in this study are evaluated separately, the methods using MM methods (diagnostic tests performed by Shimomura et al. in M0 and N0 BC cases with five microRNA panel: miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, miR-6875-5p) in the ASs take the first 4 places (A3D, A3F, A3B, A3G) (Table 3) [23]. In the study conducted by Kazi, FNB results accompanied by ultrasonography are in the 5th place in the accurate diagnosis order in BC (Figure 2) [15].

According to the findings we obtained in this study, the highest ASs in GpA were obtained in the study performed by Shimomura with the tests performed with five micro RNA panel (miR-1246, miR-1307-3p, miR-6861-5p, miR-6875-5p) [23]. Then, the most successful ASs were obtained with the triple micro RNA panel (miR-21, miR-155, miR-365) in the study by Han et al. (Figure 2) [22]. When the absolute sensitivity rates in the studies conducted in GpB is analyzed, it is seen that the highest rate was obtained in the study conducted by Kurita et al. (Figure 2) [27]. When all the studies in GpA and GpB are taken into consideration, the fifth highest success rate in the ASs appears again in this group (Figure 2). However, one of the biggest disadvantages in cases where FNB is performed for the diagnosis of BC is that the ASs decrease as the cancer mass decreases [36].

However, the size of the cancer mass does not affect the ASs in studies with MMs [23]. According to the results of

this study; even in M0 (A3G), N0 (A3F), Stage 3 (A3D), Stage 0 (A3A) and Stage 1 (A3B) cases, the highest ASs were achieved with the five-point micro RNA diagnostic panel [23]. At the same time, these results show that MM diagnostic tests in serum are very suitable to use for BC screening. According to the results obtained, in this study, the diagnostic tests with micro RNAs in BC appear to be more effective than other tests.

One of the most commonly used noninvasive screening methods for BC diagnosis in the world is mammography. In a study by Warren et al. in England; a group of women (ages 50–70 years) who had mammography every year, the risk of BC due to mammography, although it changes according to radiation dose and factors affecting the dose, has been reported as 156/1 or 312/1 [37]. This risk will be completely eliminated when MMs in serum enter routine practice in BC screening.

It is more likely that MM diagnostic methods in BC are more cost-effective than the other diagnostic methods (such as FNB and CNB). The cost-effectiveness of FNB has been investigated by MM methods used in the diagnosis of thyroid cancers by Li et al. [38]. According to the results obtained in the study, MM methods were found to be more cost-effective [38]. The limited aspect of this study, while investigating the effects of molecular marker discoveries on the diagnosis and treatment methods in BC, the cost-effectiveness could not analysed, due to the limited number of publication on this matter.

The study pointed out the general outcomes of the studies in the literature. Actually, the studies given in the present article investigated FNB and MM's sensitivities separately. Therefore, the conditions (staff, environment, patient) may differ from one study to another and thus, a concomitant comparison study conducted in the same conditions may reveal more definitive outcomes. The authors of this study believe that, although there is not enough study with serum MMs in literature and this is the first study comparing serum MMs with the risky invasive FNB and CNB methods, in the near future, serum MMs will become an indispensable in the definitive diagnosis of BC.

Conclusion

According to the results has been obtained, MMs measurement in serum is assumed as a noninvasive method that can be used safely in BC screening, definitive diagnosis and follow-up. Therefore, in the near future, serum MMs is likely to take a higher priority, in diagnostic approaches and screening programs for BC.

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