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A Survey in the Basal Like Breast Carcinoma Phenotype in North East of Iran

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Abstract

Background: Breast carcinoma is not a homogenous disease. It is divided into 5 pathological subtypes of luminal1, luminal2, strongly Her2 positive, basal like, and normal breast like. Basal like breast carcinoma that accounts for about 15% of all breast cancers has an aggressive clinical behavior with the features of high nuclear grade, negative estrogen receptor, progesterone receptor, and Her2 reactivity (triple negative).
Objectives: We aimed at identifying the prevalence of basaloid phenotype among triple negative (TN) cases in our region via immunohistochemistry (IHC) staining using basaloid markers.
Methods: We reviewed breast cancer patients in Omid and Imam Reza hospitals, Mashhad, Iran, between 2003 and 2007. We obtained the paraffin blocks from TN cases for immune-staining using cytokeratin 5/6 (CK5/6), cytokeratin 14 (CK14) and epidermal growth factor receptor1 (EGFR1) markers.
Results: The incidence of TN disease among breast cancers was 21% (156/747). Based on IHC reactivity with at least one of the basaloid markers, from 59 available samples, 44 (75.4%) were basaloid.
Conclusions: In our region, most triple negative tumors were basal like breast cancer (BLBC). Among these cases, most immunereactivity was observed for EGFR1, followed by CK14 and CK5/6.

Keywords: Breast Cancer, Basal Like, Triple Negative, Immunohistochemistry, Cytokeratin 5/6, Cytokeratin 14, EGFR1

1. Background

Breast cancer ranking first among female cancers (1), comprises 24.4% of all malignancies in women (2). Based on the international agency of research on cancer (IARC) reports (Golobocan-2008), the age standardized incidence and mortality rates of breast cancer in Iranian females were 18.4 and 8.9 per 100.000, respectively (3).

Sharifian et al. analyzed the national incidence data from the Iranian annual national cancer registration reports from 2003 to 2009 and national death statistics reported by the ministry of health and medical education from 1995 to 2010, stratified by age group. They showed that the general mortality rate of breast cancer has increased during these years from 0.96 to 4.33 per 100,000 and the incidence rate increased from 16.0 to 28.3 per 100,000 for the years under study, concluding that the burden of breast cancer for Iranian women is still increasing (4).

Breast cancer is a heterogeneous disease. According to the new classification, breast carcinomas are classified into five diverse subgroups as luminal A, luminal B, strongly Her2 positive, Basal like, and normal breast Like (5).

Basal like breast cancer (BLBC) is a specific clinical and pathological entity which consists of about 15% of breast cancers in western countries. However, according to some reports, basal-like cancer accounts for from 8% up to 37% of all breast cancers, mostly depending on the proportion of grade 3 breast cancers in their population (6, 7).

As it is generally accepted, BLBC is defined by high histological grade, predominantly triple-negativity (negative for ER, PR and Her2 receptors), basal cytokeratins expression, and a more aggressive clinical course and generally poor prognosis (8).

Almost 80% of triple negative breast cancers are basal like breast cancers. This phenotype is more common in premenopausal patients. Fifty percent of these tumors show over expression of EGFR which itself is a marker of metaplastic basal like breast cancer and an indicator of aggressive behavior. The outcome of these patients is ap-

proximately similar to ER-negative Her2-strongly positive patients. However, trastuzumab is not recommended in these cases (9, 10).

BLBC may be recognized by IHC, real-time reverse transcriptase polymerase chain reaction–based signatures (RT-PCR) or gene expression profile (GEP) methods, the latter is the gold standard for identification of BLBC. GEP is very costly and is not used in routine clinical assessments. Although RT-PCR has had a concordance rate of more than 90% with microarray based analysis, only one IHC panel has been validated by expression arrays. This panel which is comprised of ER, HER2, ck5/6 and EGFR identifies BLBC tumors with a sensitivity of 76% and specificity of 100%. The use of this immunohistochemical panel has the potential to identify a morphologic spectrum of basal-like cancer, which includes grade III invasive ductal carcinomas, atypical medullary carcinomas, and metaplastic breast cancers (10, 11).

There is still no consensus agreement on which method is proper to identify BLBC. Most authors defined BLBCs using IHC, mainly CK5 or CK5/6 reactivity in their methods. Other markers, such as CK14, CK17, EGFR, CKit, P63, P-cadherin and SMA, were also utilized (10, 11).

2. Objectives

The main objective of this study is to determine the prevalence rate of basaloid phenotype among triple negative breast cancers in our geographic region via immunohistochemistry staining for CK5, CK14 and EGFR markers and explore the criteria that can be used to identify these tumors in the routine practice.

3. Methods

We found and reviewed the documents of breast cancer patients in 2 oncology centers of Omid and Emam Reza hospitals, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, between the years 2003 and 2007. The pathological reports were searched and only those with available all basic IHC assessments (ER, PR and HER2) were included. The following criteria were selected to consider a tumor into triple negative phenotype: ER and PR were defined as negative if immunoperoxidase staining of tumor cell nuclei was less than 5% and Her2 was considered negative if IHC score was 0 or 1+. We did not encompass Her-2 moderately positive (2+), considering the requirement for negative confirmation by FISH or cytogenetic methods.

We also recorded some of demographic characteristics of the patients including age, menopausal status, histological grade of tumors (based on Bloom-Richardson grading system) and some other clinical parameters. We also repeated immune-staining for some samples for ER, PR and HER2 reactivity randomly to find if there was any discordance between original reports of triple negativity and our findings. The available samples were immunostained for basaloid markers reactivity including cytokeratin 5/6, cytokeratin 14 and EGFR proteins.

3.1. Immunohistochemistry

We collected the paraffin blocks of all the patients' biopsies, sliced and colored them with hematoxyllen-eosin. All the slides were checked by a pathologist and marked. We provided 2μ cuts from the slides. After 24 hours, they were ready for immunohistochemical staining.

At the first step, all the slides were hydrated and deparaffinated with xylon and alcohol and demaskated by EDTA-TRIS solution (PH = 7). After washing the slides with TBS (Biogene co) all of them were incubated with H2O2 and protein block (Novocastra co), and CD14 (code RTU LL002 Novocastra co), according to the markers type, for 30 minutes. Then, the slides were incubated first by post primary block and then by polymer novoline for 30 minutes (Novolink detection system REF RE7280-K). All slides were colored with chromogen DAB (Novolink detection system REF RE7280-k) and hematoxyllen. The pathologist reviewed all the slides which were dehydrated, cleaned and labeled.

The coloring method was cytoplasmic for ck5/6 and ck14 and membranous for EGFR. The reactive cell percentiles were measured as color percentage and color intensity varied between 0, +1, +2 and +3 (negative, weak, moderate and strong).

4. Results

Among 978 breast cancer cases referred to our centers during the five-year period, 231 patients were excluded due to inadequate information regarding immunohistochemistry results. From 747 remaining patients, 156 cases were ER, PR and HER2 negative. Therefore, the prevalence rate of the triple negative tumors among our study group was 21% (156/747). The mean age of these triple negative patients was 48.4 year ($\pm$ 0.997). Postmenopouse patients accounted for 47.9% of those with known menopausal status. Family history of cancer was recorded in 27.1%. Among 85 patients with a known tumor grade, 58.9% were grade 3, followed by grade 2 (37.6%) and grade 1 (3.5%). The most prevalent histological morphologies were ductal and lobular (81%, 11.7%). Women with triple negative breast cancers were slightly younger (Mean: 48 years vs. 51 years, P = 0.105, t-test), more probably premenopausal (52% vs. 47.9%: P = 0.

22), and had more commonly dedifferentiated tumors (81% vs. 19%: P = 0.000). The demographic characteristics of the 156 cases with triple negative features are illustrated in Table 1.

Table 1. Clinicopathologic Characteristics of Triple Negative Patients

Characteristics	No of Patients	%
Total enrolled	156	100
Age (years)		
Median (Range)	47.5 (25 - 79)	
Mean ± SEM	48.4 ± 0.997	
Menopausal Status		
Premenopouse	61	52
Postmenopouse	56	47.9
Unknown	39	
Familial history of cancer		
No	70	72.9
Yes	26	27.1
Unknown	60	
Histologic Grade		
Grade 1	3	3.5
Grade 2	32	37.6
Grade 3	50	58.9
Unknown	71	
Histologic Morphology		
Ductal	111	81
Medullary	16	11.7
Lobular	4	2.9%
Papillary	4	2.9
Sacomatoid	1	0.7
Metaplastic	1	0.7
Unknown	19	

We could retrieve 63 paraffin blocks of triple negative patients from which, 59 samples were proper for immunostaining. Random staining of 15 samples for ER, PR and HER2 did not show any discordance with the original pathological reports. According to our definition, 44 cases were basal like breast carcinomas. Therefore, prevalence rates of BLBC phenotype among our triple negative group and the whole study group was 74.5% and 15.6%, respectively. Among antibodies we used, EGFR turned positive the most (34 patients, 57.6%), followed by CK14 (19 patients, 32.2%) and CK5/6 (10 patients, 17%).

The frequencies of 4 different situations of immunostaining among cytogenetic markers in the study are illustrated in Tables 2 and 3.

5. Discussion

In this retrospective study consisting of 747 invasive breast cancers, 21% were triple negative. We assessed 59 proper and available triple negative samples using three basaloid IHC markers (EGFR, CK5/6, CK14) and observed the immuno-reactivity by at least one of these markers in 74% of cases, indicating a significant concordance between TNTs and BLBCs.

According to previous studies, triple negative tumors account for 6.8% to 31.7% of all breast cancers (12, 13). Compared to other breast cancer patients, the average age of this group is lower and includes more pre-menopause cases (14-19). However, one study by Iwase reported that triple negative patients were more frequently postmenopausal (16). Numerous studies indicated that in comparison with other cases, pathological grade of TNTs were higher (13, 17, 20-23) and Ki67 over expression was more common (8, 9, 14, 20, 21). In a retrospective cohort study on Korean breast cancer society registration program data, TNBC patients showed higher tumor size, lymphatic stage, histological grade, and more lymphovascular invasion. However, in multivariate analysis, only histological grade and Ki67 were significantly higher. Although TNTs are a heterogenic group of tumors. They are mostly originated from myoepithelial cells (24). This can be shown by cytogenetic studies or IHC methods; the former is more accurate, while the latter is simpler, less expensive, and more accessible.

GEP is the gold standard for the identification of basal-like breast cancer. However, the use of microarrays is expensive, largely limited to fresh or frozen samples, and difficult to use in the routine diagnostic practice. CK5 (or CK5/6) is probably the most frequently used basal CK, either alone or in combination with CK14, CK17, or both (6). Nielsen et al. reported that 62% (13/21) of basal-like tumors express CK5/6 (10). Livasy et al. demonstrated that 61% (11/18) of basal-like tumors express CK5/6 (23). Several groups have tried to define surrogate IHC markers to identify the molecular subgroups classified based on microarray expression analysis. The immunohistochemical panel comprised of 4 markers (ER, HER2, CK5/6, and epidermal growth factor receptor [EGFR]) has been validated by expression arrays and identifies basal-like cancers with 100% specificity and 76% sensitivity (10). However, this definition is not complete and has some limitations. Another approach to identify basal-like breast cancer is using triple-negative phenotype, considering that

Table 2. Basal Marker Expression of Each of Three Antibodies in 59 TNT Tumors

	EGFR (%)	CK5/6 (%)	Ck14 (%)
Negative	25 (42.4)	49 (83)	40 (68)
Weakly positive	11 (18.6)	5 (8.5)	8 (13.5)
Moderately positive	11 (18.6)	4 (6.8)	7 (11.8)
Strongly positive	12 (20.3)	1 (1.7)	4 (6.8)
Overall positive	34 (57.6)	10 (17)	19 (32.2)

Table 3. Basal Marker Expression of Each of Three Antibodies in 44 BLB Tumors

	EGFR (%)	CK5/6 (%)	Ck14 (%)
Negative	10 (22.7)	34 (77.2)	25 (56.8)
Weakly positive	11 (25)	5 (11.3)	8 (18.2)
Moderately positive	11 (25)	4 (9.1)	7 (16)
Strongly positive	12 (27.2)	1 (2.3)	4 (9.1)
Overall positive	34 (77.2)	10 (22.7)	19 (43.2)

most basal-like breast cancers are ER, PR, and HER-2 negative (the triple-negative phenotype) (10). The most practical definition of basal-like tumors is based on hormone receptors, HER2 negativity, and specific basal marker positivity (CK5/6, CK14, CK17, and EGFR) (10, 11).

Nielsen et al. added PR negativity to this definition. Based on this new definition, basal-like breast cancers are defined as ER, PR, and HER2 negative breast cancers that express CK5/6 and/or EGFR (10, 25).

Some authors believe that the addition of CK14 to this panel can improve the definition of basal-like cancer (6 markers: ER-negative, PR-negative, HER2-negative, and CK5/6-positive and/or CK14-positive and/or EGFR-positive tumors) for the following reasons: 1- CK14 is expressed in basal/myoepithelial cells of the breast and forms complexes with CK5; 2- it has a staining pattern that is more reliable than that of CK17; 3- it stains a proportion of breast cancer cases that overlap with CK5/6 positivity; and 4- it is associated with a poor outcome.

Regarding this, we used 3 markers, EGFR1, CK5/6, and CK14, which turned positive in 44%, 17% and 32% of TNTs, respectively (overall 74.5%, or 44 tumors out of 59 TNTs). According to previous studies, between 50% to 80% of triple-negative tumors express basal markers (18, 25). This subgroup of tumors is associated with poor outcome (11, 25). Our result approximated the upper limit of this range which could be due to the use of six markers in our study.

The sensitivity of each basal marker among the BLBC group was 77.2% for EGFR (34 out of 44), 43.2% for CK14 (19 out of 44) and 22.7% for CK5/6 (10 out of 44).

Some investigators have used P-cadherin (26), c-Kit (27), nestin (28), osteonectin (29), vimentin, and laminin (30, 31) to improve basal-like cancer detection. However, not all TNTs are IHC positive, because, according to GEP analysis, not all TNTs are basaloid. On the other hand, in case of IHC study of non-TNT by basaloid markers, a small percent of them are reported as positive, addressing that TNTs and basaloid cancers do not have a 100% concordance (32).

Defining the basal cell breast cancers helped us to recognize the wide spectrum of morphologies including invasive ductal carcinoma grade III, metaplastic morphologies such as spindle cell, osteoid and chonroid metaplasia, SCC, and apocrine morphology, which are more common in basaloid tumors (33, 34). In our study, we found a 46 years old triple negative metapelastic breast cancer patient. In IHC, her tumor was strongly positive for CK14 and moderately positive for EGFR, but negative for CK5/6. She found a brain metastasis by the metapelastic breast cancer with osteosarcomatose differentiation and passed away in less than one year from diagnosis. Gwin reports a series of 21 metapelastic breast cancers with chondroid differentiation that were all triple negative, with no expression of androgen receptors. Nine out of 21 cases had grade II or III DCIS. Ten cases had a very aggressive course of disease, with visceral and chest wall metastases, which led to death in 3 patients. Lymphadenopathy was very common, 60% of which had chondroid differentiation, too. EGFR1 was positive in 88%, consistent in the proposed basaloid phenotype for all metapelastic cancers (18).

Expectedly, like every other study, this one was not

without limitations. First, it was a retrospective study. Second, pathology reports and histological grading were done in different laboratories without a central review. Third, blocks were from different laboratories and different cities, and some of them lacked enough tumor or had lost quality to perform basal cell IHC on them; therefore, we could exam the paraffin blocks of only 38% (59 out of 156) of TN patient for basaloid IHC. However, our trial had advantages too. First, it contained the adequate number of breast cancer patients. Secondly, triple negativity was double checked by an expert pathologist. Third, basaloid specific IHC of all samples were performed in a central lab and reviewed by the same pathologist. Fourth, we used three specific IHC markers (overall: six markers) to enhance the sensitivity of our test; and fifth, it shows the unfavorable histological grade in TN tumors.

In conclusion, triple negative breast cancer tumors make a considerable group of breast cancers mainly from basaloid phenotype and may have de-differentiated and metaplastic features. Using different IHC markers enhances the sensitivity to detect BLBCs. This entity makes a specific subgroup of breast cancer with its specific biology, oncogenesis and clinical course. Paying attention to this specific category, the clinician may take advantage of specific treatments in the future.

5.1. Conclusion

The great majority- but not all - of TNTs are BLBC. In our study, the most immunopositivity in IHC staining was observed by EGFR1, followed by CK14 and CK5/6. Screening for basaloid breast cancer in TNTs may be useful in deciding for therapeutic strategy.

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Footnotes

Authors' Contribution: Roham Salek and Azar Fanipakdel have designed the present study; Roham Salek, Fatemeh Varshoee Tabrizi, Kamran Ghaffarzadegan, and Azar Fanipakdel have written the article; Roham Salek and Azar Fanipakdel have edited the article; Fatemeh Varshoee Tabrizi, Kamran Ghaffarzadegan, Golnaz Sabouri and Azar Fanipakdel have been responsible for collecting the data; Roham Salek, Kamran Ghaffarzadegan, and Azar Fanipakdel have contributed to the analysis and data interpretation.

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