Male Invasive Breast Carcinoma: Nine Years Experience at a Single Center in Turkey

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Synopsis: However there are huge published articles breast carcinoma in female population, information on male group that particular subject is globally restricted especially from the developing countries. Here, we aimed to present our results from a large institution in Turkey.

ABSTRACT

Objective: Male breast cancer (MBC) is rarely seen in male sex with a prevalence in the general population of approximately 1 per 100,000. Our aim was to determine retrospectively the clinicopathological features and overall survival at 22 male invasive breast carcinoma cases with the molecular sub-types based on immunohistochemistry in nine years period.

Methods: All male patients presenting with invasive breast carcinoma between January 2006 and September 2014 data were recorded regarding age, clinical presentation, operative procedure, tumor size, histologic type, grade, lymph node involvement, immunohistochemical results of HER-2, hormone receptors, Ki-67 and p53 with stage and outcome.

Results: The mean age of the group was 68.05 years with a 72.7% had performed a modified radical mastectomy (MRM). The mean diameter of tumors was 2.63 cm and the most common histologic type was invasive ductal carcinoma (IDC). Notably there was no grade I tumor and Luminal B type had slightly high in number.

Conclusion: We found a significant correlation in between luminal molecular subtype and Ki67 proliferation. The mean overall survival time was 75.3 months. Luminal subtypes were not showed significant difference with overall survival time.

Keywords: malebreastcarcinoma, molecularsubtype, immunohistochemistry

INTRODUCTION

Male breast cancer (MBC) is a rarely seen in male gender with a prevalence in the general population of approximately 1 in 100,000 and less than 0.5% of all cancer deaths in men (1,2). In general for understanding of tumor carcinogenesis have been performed many molecular studies. In breast carcinomas first molecular study was performed in 2000 (3). At that very beginning the suggested new intrinsic molecular classification was as Luminal A, Luminal B, ErbB2 overexpression, basal-like and normal-like types based on gene expression analysis on DNA microarrays; however nowadays for practical reasons immunohistochemistry is the first choice method of molecular subtyping.

This approaching was supported in St. Gallen International Breast Cancer Conference 2011 using a panel of five biomarkers including estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor 2 (EGFR2), HER2/neu (Cerb-B2) and Ki67 proliferation index (4). Indeed, the studies to date have been proved the importance of molecular subtyping in breast cancer hides under its capability on the prognosis prediction. Our goal was to investigate retrospectively the clinicopathological features and overall survival at 22 male invasive breast carcinoma cases with the molecular sub-types based on immunohistochemistry.

METHODS

This is a retrospective study of all male patients presenting with invasive breast carcinoma between January 2006 and September 2014 data was recorded regarding age, clinical presentation, operative procedure, tumor size, histologic type, grade, lymph node involvement, immunohistochemical results of HER-2 including hormone receptors, Ki-67, p53 with stage and outcome. Follow-up time was defined as in months from diagnosis time to

the final visit. Histologic type, grading and staging were characterized using the tumor classification set by the World Health Organization (5).

Tissue sections were re-evaluated under light microscopy and the most representative tissue selected if needed. For immunohistochemical (IHC) stains the cut sections (thickness with 4–5 microns) were taken from the paraffin-embedded tumor tissues and set on the glass slides made of poly-lysine. Tissue sections on glass slides were kept in an incubator at 60°C overnight, deparaffinized, and rehydrated in a graded series of alcohol. Deparaffinized slides were incubated in a Dako PT Link system in a preliminary procedure (Dako, Glostrup, Denmark) using an EnVision FLEX Target Retrieval Solution (high pH; Dako). The solution was heated 95°C and incubated for 20 min, then cooled down to 65° C. Sections were loaded onto an Autostainer (Dako) for IHC staining.

The following solutions and reagents were administered: EnVision FLEX Washing Buffer, EnVision FLEX Peroxidase-Blocking Reagent (incubated for 5 min), primary antibody estrogen receptor (ER – monoclonal rabbit EP1 clone), progesterone receptor (PR – monoclonal mouse PR636 clone), c-erbB-2 polyclonal rabbit oncoprotein, Ki-67 (monoclonal mouse MIB-1 clone), p53 (monoclonal mouse DO-7 clone). All antibodies are ready to use and were provided from DakoCytomation (Denmark). The external control slides were used when needed. EnVision FLEX/Horseradish Peroxidase (incubated for 20 min), Envision FLEX 3,3 diaminobenzidine tetrahydrochloride Working Solution (incubated for 10 min) and hematoxylin (for counterstaining).

After immunostaining sections were put into graded alcohol (80, 96, 99 %). Xylol was used to make sections transparent, and they were coated with balsam for examination. In consider to determining of the antibody distribution pattern, percentage of positive cells and intensity of reactive tumor cells were scored semiquantitatively for ER and PR. A positive result was considered if at least 1% of cells nuclear expression (6).

HER-2 (Cerb-B2) was scored using the new recommendations of *ASCO/CAP Guidelines*, and was quantified as follow: 0 if no membrane staining is observed in invasive tumor cells; 1+ if is observed weak, incomplete membrane staining in any proportion of invasive tumor cells, or weak, complete membrane staining in less than 10% of cells; 2+ for complete membrane staining that is non-uniform or weak but with obvious circumferential distribution in at least 10% of cells, or intense complete membrane staining in 30% or less of tumor cells; 3+ if is seen strong and uniform staining of the entire membrane in more than 30% of cells. Cases with immunohistochemically 2+ score (equivocal) were further analyzed for HER2 gene amplification by fluorescence *in situ* hybridization (FISH) technique (7).

Percentage of Ki67 immunostaining was meant to the nuclear stained cell with a value of 14% using as a cut-off value for low or high expression (8, 9). p53 was considered positive whether more than 10% of tumor nuclei were stained. In regard to immunohistochemical expressions of ER, PR, HER2 and Ki67 the tumor was classified into following molecular subtypes: Luminal A (ER+ and/or PR+, HER2-, low Ki67), Luminal B (ER+ and/or PR+, HER2+, any Ki67 or ER+ and/or PR+, HER2-, high Ki67), HER2+/ER- (ER-, PR-, HER2+), triple negative (ER-, PR-, HER2-) (3,10).

Statistical analysis was performed using *StatisticalPackage for Social Sciences* (SPSS) software program, version 20.0. Descriptive statistics were calculated, providing mean and standard deviation for continuous variables and frequency (percentage) for categorical variables. The association between continuum variable was done using independent Student's *t*-test and for categorical variable was established by Fisher's exact test. Kaplan–Meier analysis was performed to calculate survival curves and *log*-rank test was used to assess the statistical significance of the differences between IHC subtypes. A two-tailed *p*-value <0.05 was considered significant.

RESULTS

In between, January 2006 and August 2014 out of 104 surgical specimens of male breast 22 cases had been reported as invasive breast carcinoma (21.1%). While 45.5% of the tumors were in-house cases 54.5% was consultation cases. The age of the patients range from 54 to 91 year-old with a mean 68,05 and median 67.5 A modified radical mastectomy (MRM) were performed 16 of 22 patients 72.7%, 3 breast conservative surgery (BCS) 13.6 %, 1 incisional biopsy 4.5%, 2 patients of the series were not recorded in the files.

Right breast was more affected (63.6%) than left (36.4%). All of the recorded cases were localized in the retroareolar region (100%). Mean tumor diameter was 2,63 cm (median 2,75) cm (range from 0.5 cm to 5 cm). One case had two foci of tumor each 0.5 mm (case no 22). Invasive ductal carcinoma (IDC) was the most frequent form 14/22, 63.6%. 5/22 cases were represented by mixed type breast carcinomas (MTBC) 22.7%, 2/22 invasive papillary carcinoma (IPC) 9%, 1/22 purely invasive micropapillary carcinoma (IMPC), 4.5%. IMPC was the most frequent component in MTBCs, as well (Figure 1). There was no grade 1 cases in this current series, the most frequent cases (18/22) were classified to grade 2 (81.8%), 4/22 cases were recorded grade 3; 18.1%. In recorded files 15 cases with axillary dissection, lymph node involvement was identified in eight (53.3%) and the mean number of the dissected node was 14.6.

In addition, two cases showed intramammarian lymph node metastasis. ER positivity detected in 21 cases (95.4%), PR positivity in 19 (86.3%). Cerb-B2 score 3 was in three cases (13.6%). P53 gene expression noted in 8 (%36.3). Ki67 score was high in 9 (%40.9) (Figure 2). While luminal A type tumors were in 10 cases (45.4%) luminal B (50%) types were in 11, one case was triple negative (4.5%) (Tables 1-2). Only one patient who was triple negative was excluded to detailed statistical analysis. Table 3 is the main clinicopathological features of MBC correlated with luminal molecular subtypes. We found a significant correlation only

in between luminal molecular subtype and Ki67 index but no significant correlation with age, tumor diameter, hormonal status, tumor type, grade, nodal status and stage, CerbB-2 and p53.

The numbers of stage I-II and stage III-IV were 15 (68.1%), 5 (22.7%), respectively. Two cases were not recorded in database. The mean follow-up time was 16.6 months (1-78) in 21 patients, one case was missed in files. The four cases had distant metastasis including bone and lung. Out of three cases two were died of disease. Since only 3 of 22 patients died median survival was not reached. The mean overall survival time was 75.3 months Conventional therapeutic approaches were performed to the patients including radiotherapy and/or chemotherapy with hormonotherapy except the case that the only case of the series who was DOD in one month. A significant correlation was noted in between overall survival with ER and PR, p= 0.003, p<0,001, respectively. Neither significant difference was determined p53, C-erbB-2, and Ki67 p= 0,981 p=0,461, p=0.35 respectively, nor luminal subtype was determined in overall survival time (p=0,343) (Figure 3).

DISCUSSION

MBC makes up less than 1% of all cancers in men and less than 1% of all breast cancers in the United States (2). According to our institutional files male breast cancer approximately was 1.6% of all invasive breast cancers in both sexes. MBC is seen 6th decade in different studies the mean age was 68,05 at diagnosis in our series (11). Similar to the English literature right breast involvement was slightly increased than left breast (63.6%) and all of the recorded cases were localized in the retroareolar region (100%), the mean tumor diameter was 2.6 cm (12-15). One patient of the group had 2 foci of tumor, to our knowledge this was the first multifocal case within the published series. IDC was the most frequent form 63.6% which has been slightly lower than the other series published (75.4% to 90.4) (12-18).

Although the percentage was the highest among those published series before, the majority of the cases (18/22) were classified to grade 2 (81.8%), as well (12,17,19). Furthermore, there was no grade 1 tumor in the current series. It can be speculated for the lower ratio of the IDC versus mixed types.

Nodal involvement varies from 20.7% to 77.2% (8,12,14,17-21). Almost half of the cases showed lymph node metastasis, 53.3%, in our series. Comparing to the previous reported series, luminal A molecular subtype showed a decreased percentage 45.4% in the study (15,18,21).

Additionally, stage I-II group varies from 38 to 58% in English literature, we noted a higher percentage in early stages, 68.1% (12-16).

As opposed to the study from Aschie et al., Luminal B tumors were slightly frequent in our series with an insignificant outcome than Luminal A subtype (15).

CONCLUSION

We found a significant correlation in between luminal molecular subtype and Ki67 proliferation. Also, a significant correlation was noted in between overall survival and hormon receptors. Our study showed that molecular Luminal subtypes are almost equal with an only one triple negative tumor and no HER2 type. The number of patients is not enough for a concise foresight, at least our results might be symbolized including histologic and molecular subtypes of the MBC with clinical information from a tertiary hospital in our country.

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Table 1: Demographic and histopathologic findings of the group R: right, L: Left, RA: Retroareolar

Case No	Age	Side/Location	Surgical Procedure	Size (cm)	Diagnosis	Grade	Lymph nodes
1	61	R/RA	MRM	1,5	IDC	II	3/21
2	77	R/RA	MRM	3	IDC	II	4//6
3	79	L/x	unknown	2,5	IDC	II	X
4	77	L/x	MRM	3,5	IDC	II	x
5	75	R/x	unknown	0	IDC	II	x
6	57	R/x	Incision	3	IDC	II	x
7	79	R/RA	MRM	1,5	IDC	II	0/7
8	61	R/RA	MRM	4	IDC	II	3/22
9	69	L/x	MRM	1,5	MIXED (IDC+MUC+IMPC)	II	0/2
10	82	L/RA	MRM	5	IDC	III	8/19
11	54	x/x	BCS	1,5	IDC	II	0/8
12	57	R/RA	MRM	3,5	MIXED (ILC+IMPC)	II	9/17
13	91	R/x	MRM	5	MIXED (IDC+IMPC)	III	6/6
14	59	R/x	BCS	2	IDC	III	x
15	61	R/RA	MRM	3	IDC	III	0/17, 2/2
16	75	R/x	MRM	3	MIXED (ICC+IMPC)	II	x
17	69	L/RA	MRM	2	IPC	II	0/16
18	66	L/x	BCS	5	MIXED (IDC+IPC)	II	x
19	58	R/RA	MRM	3,8	IDC	II	2/12 0/1
20	70	L/RA	MRM	1,5	IPC	II	0/20
21	60	R/x	MRM	2	IDC	II	2/11
22	60	R/RA	MRM	*0,5	IMPC	II	0/36

Table 2: IHC results. H: High, L: Low, U: Unknown

Case No	ER	PR	cerbB-2	p53	Ki67	Molecular Subtype
1	(+)	(+)	(-)	(-)	H	Luminal B
2	(+)	(+)	(+)	(-)	L	Luminal B
3	(-)	(-)	(-)	(+)	L	Triple (-)
4	(+)	(+)	(-)	(-)	L	Luminal A
5	(+)	(+)	(+)	(-)	U	Luminal A
6	(+)	(-)	(-)	(-)	U	Luminal A
7	(+)	(-)	(-)	(-)	L	Luminal A
8	(+)	(+)	(-)	(+)	H	Luminal B
9	(+)	(+)	(+)	(+)	H	Luminal B
10	(+)	(+)	(-)	(-)	L	Luminal A
11	(+)	(+)	(-)	(+)	L	Luminal A
12	(+)	(+)	(-)	(+)	H	Luminal B
13	(+)	(+)	(-)	(+)	H	Luminal B
14	(+)	(+)	(-)	(-)	L	Luminal A
15	(+)	(+)	(-)	(+)	H	Luminal B
16	(+)	(+)	(-)	(+)	L	Luminal A
17	(+)	(+)	(-)	(-)	L	Luminal A
18	(+)	(+)	(-)	(-)	H	Luminal B
19	(+)	(+)	(-)	(-)	L	Luminal A
20	(+)	(+)	(-)	(-)	L	Luminal A
21	(+)	(+)	(-)	(-)	H	Luminal B
22	(+)	(+)	(-)	(-)	H	Luminal B

Table 3: Analysis of the main clinicopathological features of invasive MBC in molecular subtypes according to IHC. n: number, LN: lymph node

	Total n (n=22)	Luminal A (n=10)	Luminal B (n=11)	Triple negative (n=1)	P value
Mean age	68.05 (54-91)	67 (54-82)	68.9 (57-91)	70	0,841
Mean size (cm)	2.6(0,5-5)	2,6 (1,5-5)	3 (0.5-5)	2,5	0,903
LN (+)	9 (%40.9)	2 (%20)	7 (%63.6)		0,537
LN (-)	8 (%36.3)	6 (%60)	2 (%18.1)		0,645
Unknown	5 (%22.7)	2 (%20)	2 (%18.1)	1	0,921
Grade II	18 (%81.8)	8 (%80)	9 (%81.8)	1	0,901
Grade III	4 (%18.1)	2 (%20)	2 (%22.2)		0,901
Stage I-II	15 (%68.1)	7 (%70)	7 (%63.6)	1	0,765
Stage III-IV	5 (%22.7)	2 (%20)	3 (%27.2)		0,921
Unknown	2(%9.09)	1(%10)	1 (%9.09)		0,921
ER (+)	21 (%95.4)	10 (%100)	11 (%100)		0,343
ER (-)	1 (%4.5)	0 (%0)	0 (%0)	1	0,343
PR (+)	19 (%86.3)	8 (%80)	11(%100)		0,168
PR (-)	3 (%13.6)	2 (%20)	0	1	0,131
HER2 (+)	3 (%13,6)	0 (%0)	3 (%27.2)		0,082
HER2 (-)	19 (% 86.3)	10 (%100)	8 (%72.7)	1	0,082
Ki 67 high	9 (%40.9)		9 (%81.87)		0,003
Ki 67 low	11 (%50)	9 (%90)	1 (%9.09)	1	0,002
Unknown	2 (%9.09)	1 (%10)	1 (%9.09)		0,947
P53 (+)	8 (%36.3)	2 (%20)	5 (%45.4)	1	0,233
P53 (-)	14 (%63.3)	8 (%80)	6 (%54.4)		0,233

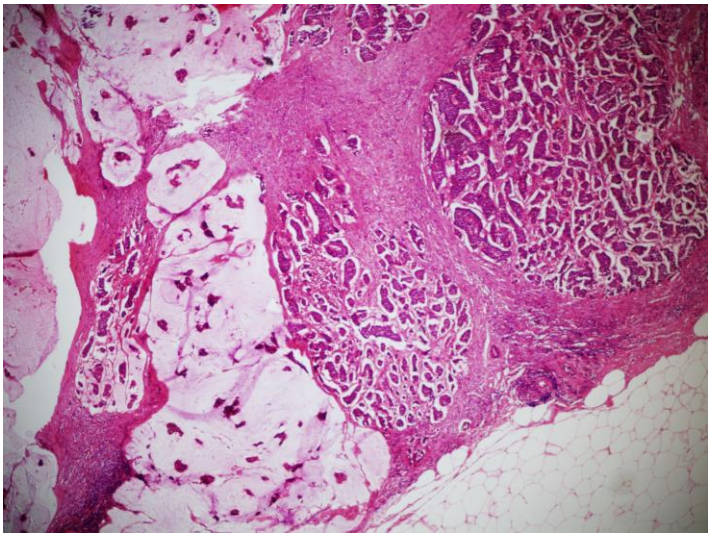

Fig. 1. : Invasive micropapillary and mucinous carcinoma containing mixed type breast carcinoma. Hematoxyline and eosin X4.

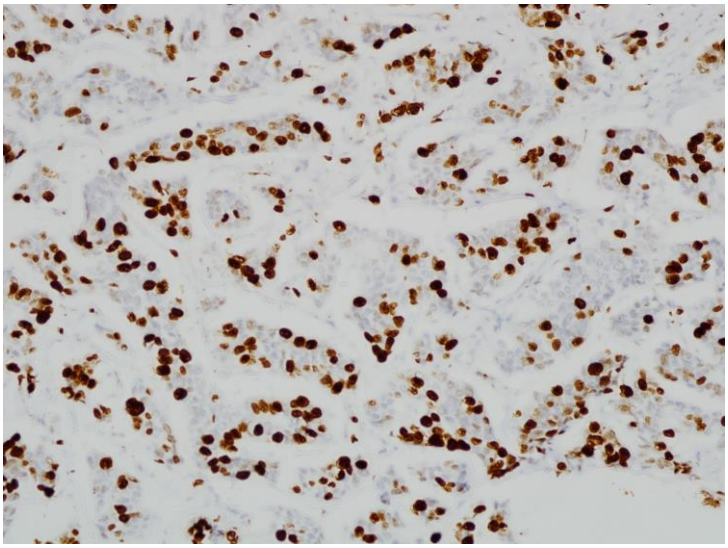

Fig. 2: A tumor showing high Ki67 proliferation index X20.

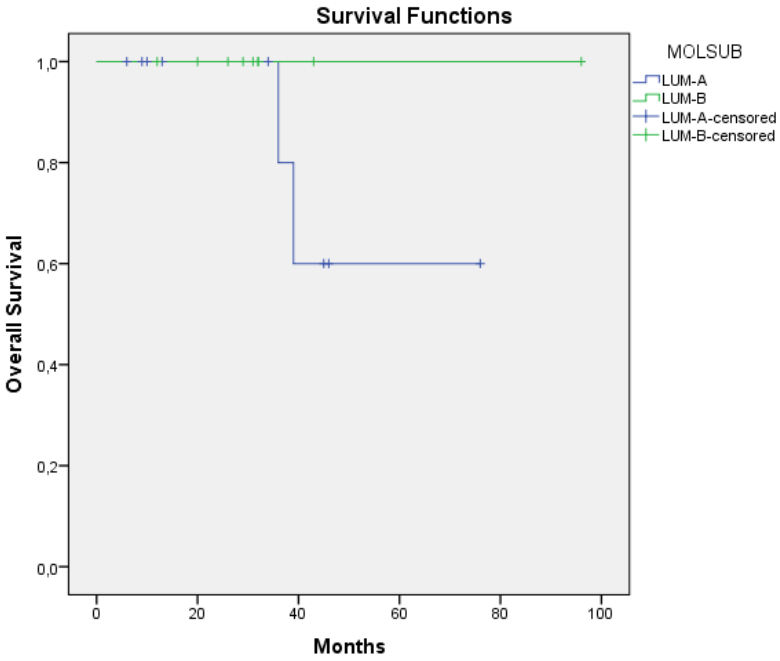

Fig. 3: Kaplan–Meier survival plot for MBC categorized according to molecular Luminal subtypes.