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HIGH GRADE BREAST DUCTAL CARCINOMAS HAVE HIGH DENSITY OF TUMOR-ASSOCIATED MACROPHAGES

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***Background*: The role of tumor-associated macrophages (TAMs) is double-natured and still controversial. Depending on different settings, macrophages may suppress or promote tumor growth. TAM density may be one of the predictive factors of treatment outcome in cancer patients. *Aim*: To evaluate the density of tumor-associated macrophages in breast cancer and its relationship with various histopathologic findings. *Materials and Methods*: 55 patients with invasive ductal carcinoma of breast who underwent mastectomy were enrolled. Sections of tumor samples were stained and the density of $CD68^+$ cells was evaluated. *Results*: There was an association between estrogen receptor (ER) expression and CD68 density ($p = 0.010$) as the higher densities of CD68 were seen in ER negative tumors. Moreover, there was a significant relationship between histological grade and CD68 density ($p = 0.006$). *Conclusion*: The higher TAM density is associated with higher tumor grade and negative ER expression in breast cancer tissues. These findings revealed that inflammation could have an important role in malignancies.**

***Key Words*: breast cancer, tumor grade, inflammation, macrophage.**

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Breast cancer (BC) is the most common cancer among women worldwide. Despite the increasing incidence rate, its mortality rate is decreasing. It accounts for 32% of women's malignancies and the mortality rate is about 15% [1, 2].

BC comprises ductal and lobular types divided into four subgroups according to stromal invasion: ductal carcinoma *in situ*, lobular carcinoma *in situ*, invasive ductal carcinoma and invasive lobular carcinoma [3] and it is characterized by histopathologic, clinical and molecular phenotype heterogeneity [4]. Microscopically, malignant tumor consists of proliferating cancer cells and microenvironment; the latter consisting of endothelial cells, fibroblasts, inflammatory cells (e.g. macrophages) and extracellular matrix. Macrophages may account for about half of tumor mass and represent heterogeneous cellular population [5].

There are different factors which can affect prognosis and survival of the patients and among which tumor metastasis is one of the most important one [6]. Metastasis occurs through epithelial mesenchymal transition, angioinvasion, tumor cells circulation and immigration, mesenchymal epithelial transition and finally colonization of tumor cells [6].

Based on literature, macrophages can take part as a tumor suppressor or promoters of tumor growth and metastasis [7]. Also, it was shown that tumor associated macrophages (TAM) could contribute to angiogenesis, matrix transformation and immunosuppression which are main steps of tumor metastasis [8]. Therefore, the recent studies on BC have revealed a probable relationship between TAMs and poor prognosis [6].

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Abbreviations used: BC – breast cancer; ER – estrogen receptor; HER2 – human epidermal growth factor receptor 2; HPF – high power field; IHC – immunohistochemistry; PR – progesterone receptor; TAM – tumor-associated macrophage.

Recent studies have shown that TAMs could promote epithelial mesenchymal transition in different ways [9], for example by growth arrest specific gene ((GAS6)/Axl) pathway & (nuclear factor-kB) in squamous cell carcinoma of oral cavity [10]. In addition, it has been shown that TAMs can induce stem-cell like features in hepatocellular carcinoma through TGF-β1 pathway [6] and increase invasiveness of renal cell carcinoma through AKT/mTOR signaling pathway [11].

In this study, we aimed to evaluate the amount of macrophages in BC and its relationship with various histopathologic findings.

MATERIALS AND METHODS

55 consecutive patients, mean age of 49.6 ± 10.6 years (range 29–72 years), who were diagnosed with invasive ductal carcinoma of breast and underwent mastectomy were enrolled. The study was approved by the Ethics Committee of Urmia University of Medical Sciences.

Paraffin blocks taken from the archive of pathology department were stained with hematoxylin and eosin and immunohistochemical (IHC) staining of CD68 was done. The prepared glass slides were reinvestigated and tumor grading and staging were performed according to Nottingham modification of Bloom Richardson system and the American Joint Committee on Cancer system. According to TNM (American Joint Committee on Cancer) staging scoring system one of the evaluated patients was at stage I, 32 of them were at stage II and 22 were at stage III. IHC staining of these samples for hormone profile including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu)and Ki67 were also included.

Sections of 4 micron thickness were obtained from paraffin embedded blocks and IHC staining for CD68 marker was done according to manufacturer's instructions. A sample from a lymph node was used as a positive control with diffuse cytoplasmic

staining pattern. Ready-to-use CD68 antibody clone PG-M1 and associated reagents were obtained from DAKO Corporation, Denmark.

IHC staining results for CD68 marker were evaluated as follow (known as Gwak method) [12]. The areas of maximum density for TAMs were determined by ×100 magnification; then the average of CD68 positive cells counted in 3 fields of × 400 magnification (high power field — HPF) was considered as the density of TAMs. The slides were evaluated by light microscopy (Olympus, Japan).

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., USA). The normality of data was evaluated with the Kolmogorov — Smirnov test. Numeric data were reported as Mean ± standard deviation and nonparametric data were reported as mean ± standard error of mean). The qualitative data were determined by $\chi 2$ analysis. The quantitative data was performed using Student's *t*-test & ANOVA and *P*-values $\leqslant 0.05$ were considered as statistically significant.

RESULTS

The mean of TAM density was 31.92 ± 3.4 (mean ± standard error of mean) with median of 25 (Table). The lowest density of CD68 antigen in this study was 3/HPF and the maximum density was 100/HPF (Fig. 1 and 2). According to statistical median of TAM density, the cases were divided into two (high & low density) groups. Densities $\leqslant$ 25/HPF were considered as low and > 25/HPF as high density. So, 26 (47.3%) cases were in low density group and 29 (52.7%) were in high density group.

The relationship between different characteristics of tumor and TAM density is given in the Table. There was an association between ER expression and CD68 density ($p = 0.01$) as the higher densities of CD68 were seen in ER negative tumors. Moreover, there was a statistical significant relationship between histological grade and CD68 density ($p = 0.006$) as the higher the histological grade, the higher the density of CD68. No association was found between CD68 density and lympho-vascular invasion, perineural invasion, nipple involvement, skin involvement, axillary lymph node involvement, clinical stage, age, PR and Her2 expression.

Table. Characteristics of evaluated tumors and macrophage density

Characteristics of evaluated tumors		Macrophage density, n (%): low	high	*P*-value
Histologic grade	Grade 1	1 (3.8)	1 (3.4)	0.006*
	Grade 2	19 (73.1)	9 (31.1)	
	Grade 3	6 (23.1)	19 (65.5)	
Lymphovascular invasion	Present	20 (76.9)	21 (72.4)	0.764
	Absent	6 (23.1)	8 (27.6)	
Perineural invasion	Present	11 (42.3)	9 (31.0)	0.386
	Absent	15 (57.7)	20 (69.0)	
Nipple involvement	Present	5 (19.2)	5 (17.2)	0.849
	Absent	21 (80.8)	24 (82.8)	
Skin involvement	Present	5 (19.2)	7 (24.1)	0.660
	Absent	21 (80.8)	22 (75.9)	
Axillary lymph node involvement	Present	6 (23.1)	6 (20.7)	0.831
	Absent	20 (76.9)	23 (79.3)	
ER expression	Present	20(36.36)	10(18.18)	0.010*
	Absent	9((16.37)	16(29.09)	
PR expression	Positive	18 (69.2)	13 (44.8)	0.071
	Negative	8 (30.8)	16 (55.2)	
Her2 expression	Positive	10 (38.5)	11 (37.9)	0.968
	Negative	16 (61.5)	18 (62.1)	

Note: *P-values < 0.05 are statistically significant.

DISCUSSION

BC is the most common cancer among women worldwide and the leading cause of death among females [13]. Although BC incidence is lower in Iran compared to other countries, its incidence and mortality rates are increasing [14]. In our study, more than 30% of patients were under 30 years in contrast to only 6% reported in literature [3].

Inflammation has an important role in cancer development, metastasis and resistance to chemotherapy. TAMs represent significant component of inflammation and have a significant role in tumor progression and metastasis [15].

Consistent study of Zhang *et al.* [16] has shown that CD68 positive TAM density had no relationship with age, tumor size, menopause, lymph nodes involvement, ER and PR expression but they found a relation-

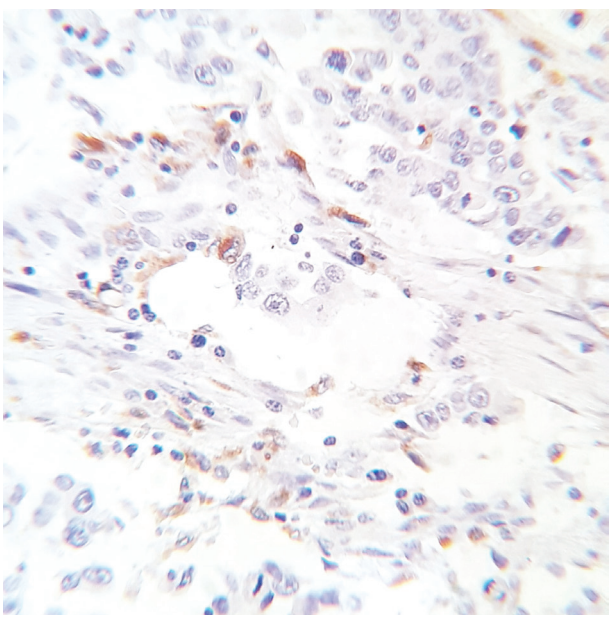

Fig. 1. IHC staining of tumor tissue for CD68 showing low macrophage density, ×40

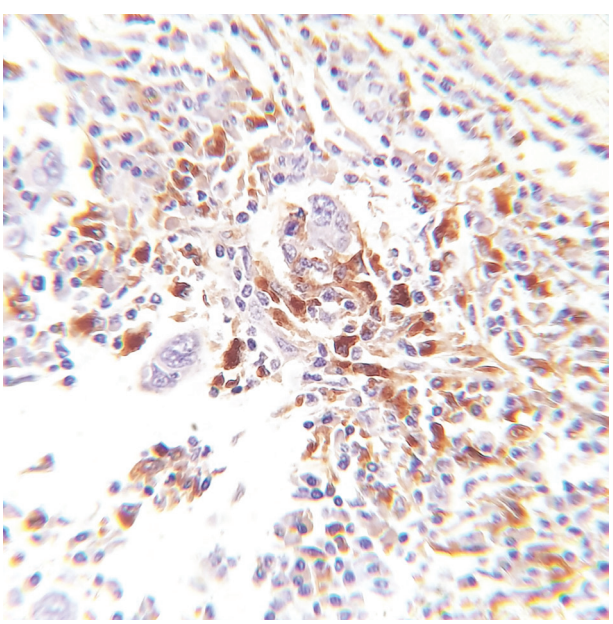

Fig. 2. IHC staining of tumor tissue for CD68 showing high macrophage density, ×40

ship between tumor histologic grade and TAM density, as the higher histologic grade (grade 3) the higher density of CD68 were seen, similar to our findings, and the average density of CD68 positive macrophages was 26±13.6 (1–80/HPF). In the studies by Campbell *et al*. [17, 18], a significant relationship between TAM and histological grade, ER and PR expression was similar to our findings. Morita *et al.* [15] also demonstrated a relationship between TAMs and ER receptor expression that was similar to our study.

In fact, our study had some limitations. It was a retrospective one and we could not evaluate the relationship between TAM and patients' prognosis and survival. Although it was not our scope, but evaluating the underlying mechanisms of interaction between macrophages and tumor cells and exploring the corresponding cytokines would give us a better view to understand the role of inflammation in BC and could help to improve the therapeutic approaches and subsequently patients' outcome and prognosis. In this study, we did not evaluate the M1 and M2 macrophages separately. Further studies to evaluate the role of M1 and M2 macrophages separately, and also their correlation with BC characteristics would give a better understanding of the role of macrophages in this cancer type.

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REFERENCES

1. World Health Organization. Global cancer observatory. Cancer today. Available from: https://gco. iarc. fr//(accessed on 23 Oct, 2020). 2019

2. **Taghavi A, Fazeli Z, Vahedi M, *et al***. Increased trend of breast cancer mortality in Iran. Asian Pac J Cancer Prev 2012; **13**: 367–70. doi: 10.7314/apjcp.2012.13.1.367

3. Rosai and Ackerman's Surgical Pathology. NewYork: Elsevier India, 2011. 2892 p.

4. **Zhao X, Qu J, Sun Y, *et al***. Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. Oncotarget 2017; **8**: 30576–86. doi: 10.18632/oncotarget.15736

5. **Tiainen S, Tumelius R, Rilla K, *et al***. High numbers of macrophages, especially M2-like (CD163-positive), correlate with hyaluronan accumulation and poor outcome in breast cancer. Histopathology 2015; **66**: 873–83. doi: 10.1111/his.12607

6. **Fan QM, Jing YY, Yu GF, *et al*.** Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. Cancer Lett 2014; **352**: 160–8. doi: 10.1016/j.canlet.2014.05.008

7. **Klingen TA, Chen Y, Aas H, *et al***. Tumor-associated macrophages are strongly related to vascular invasion, non-luminal subtypes, and interval breast cancer. Hum Pathol 2017; **69**: 72–80. doi: 10.1016/j.humpath.2017.09.001

8. **Mantovani A, Schioppa T, Porta C, *et al***. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 2006; **25**: 315–22. doi: 10.1007/s10555-006-9001-7

9. **Zhang WJ, Wang XH, Gao ST, *et al***. Tumor-associated macrophages correlate with phenomenon of epithelial-mesenchymal transition and contribute to poor prognosis in triple-negative breast cancer patients. J Surg Res 2018; **222**: 93–101. doi: 10.1016/j.jss.2017.09.035

10. **Lee CH, Liu SY, Chou KC, *et al***. Tumor-associated macrophages promote oral cancer progression through activation of the Axl signaling pathway. Ann Surg Oncol 2014; **21**: 1031–7. doi: 10.1245/s10434-013-3400-0

11. **Yang Z, Xie H, He D, Li L.** Infiltrating macrophages increase RCC epithelial mesenchymal transition (EMT) and stem cell-like populations via AKT and mTOR signaling. Oncotarget 2016; **7**: 44478–91. doi: 10.18632/oncotarget.9873

12. **Gwak JM, Jang MH, Kim DI, *et al***. Prognostic value of tumor-associated macrophages according to histologic locations and hormone receptor status in breast cancer. PLoS One 2015; **10**: e0125728. doi: 10.1371/journal.pone.0125728

13. **Tang X**. Tumor-associated macrophages as potential diagnostic and prognostic biomarkers in breast cancer. Cancer Lett 2013; **332**: 3–10. doi: 10.1016/j.canlet.2013.01.024

14. **Mousavi SM, Montazeri A, Mohagheghi MA, *et al***. Breast cancer in Iran: an epidemiological review. Breast J 2007; **13**: 383–91. doi: 10.1111/j.1524-4741.2007.00446.x

15. **Morita Y, Zhang R, Leslie M, *et al***. Pathologic evaluation of tumor-associated macrophage density and vessel inflammation in invasive breast carcinomas. Oncol Lett 2017; **14**: 2111–8. doi: 10.3892/ol.2017.6466

16. **Zhang Y, Cheng S, Zhang M, *et al***. High-infiltration of tumor-associated macrophages predicts unfavorable clinical outcome for node-negative breast cancer. PLoS One 2013; **8**: e76147. doi: 10.1371/journal.pone.0076147

17. **Campbell MJ, Tonlaar NY, Garwood ER, *et al***. Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome. Breast Cancer Res Treat 2011; **128**: 703–11. doi: 10.1007/s10549-010-1154-y

18. **Campbell MJ, Wolf D, Mukhtar RA, *et al***. The prognostic implications of macrophages expressing proliferating cell nuclear antigen in breast cancer depend on immune context. PLoS One 2013; **8**: e79114. doi: 10.1371/journal.pone.0079114

ПРОТОКОВИЙ РАК ГРУДНОЇ ЗАЛОЗИ ВИСОКОГО СТУПЕНЮ ЗЛОЯКІСНОСТІ ХАРАКТЕРИЗУЄТЬСЯ ВИСОКОЮ ЩІЛЬНІСТЮ ПУХЛИНОАСОЦІЙОВАНИХ МАКРОФАГІВ

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Стан питання: Роль пухлиноасоційованих макрофагів є двоїстою і суперечливою. Залежно від конкретної ситуації макрофаги можуть як пригнічувати ріст пухлини, так і спричиняти його. Щільність пухлиноасоційованих макрофагів може бути одним із предиктивних факторів результатів лікування онкологічних хворих. ***Мета***: Визначити щільність пухлиноасоційованих макрофагів у тканині раку грудної залози різної гістологічної структури. ***Матеріали та методи***: У дослідження включено 55 хворих з інвазивним протоковим раком грудної залози, яким було виконано мастектомію. У зрізах операційного матеріалу визначали щільність CD68$^+$ клітин. ***Результати***: Показана асоціація між експресією рецептора естрогену та щільністю CD68 (p = 0,010), найвища щільність спостерігалася в пухлинах, негативних за рецептором естрогену. Продемонстровано також достовірну залежність між щільністю CD68 та ступенем диференціювання клітин раку грудної залози (p = 0,006). ***Висновки***: Вища щільність пухлиноасоційованих макрофагів пов'язана з низьким ступенем диференціювання пухлинних клітин та відсутністю експресії рецептора естрогену. Одержані результати свідчать про важливу роль факторів запалення в пухлинному процесі.

Ключові слова: рак грудної залози, ступінь диференціювання, запалення, макрофаги.

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