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Research Article

ROLE OF CIRCULATING AND TUMOR ASSOCIATED IMMUNE CELLS INFILTRATION IN TRIPLE NEGATIVE BREAST CANCER (TNBC) AND THEIR ASSOCIATION WITH INFLAMMATORY AND HYPOXIC MARKERS

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ABSTRACT

Aim: To study the role of circulating and tumor associated immune cells infiltration in Triple Negative Breast Cancer (TNBC) and their association with inflammatory and hypoxic markers.
Methods: In this study, 50 Triple Negative Breast Cancer (TNBC) patients and 25 healthy controls were enrolled. Of them, differential WBC count was available in only 34 TNBC patients. Neutrophil count, Lymphocyte count, Neutrophil to Lymphocyte ratio (NLR) and Platelet to Lymphocyte ratio (PLR) was studied in these 34 TNBC patients and compared with healthy controls. CD3+ T cells, MPO+ Neutrophils as well as hypoxic markers IL8, HIF-1and TNF-α were evaluated in all 50 TNBC patients by immunohistochemistry (IHC) method.
Results: In comparison to healthy controls, mean percentage of neutrophils and NLR was significantly higher in TNBC patients, whereas mean percentage of lymphocytes (p=0.002) was significantly lower in TNBC patients. In relation to clinicopathological variables, increased neutrophils, high NLR and decreased lymphocytes were associated with large tumor size, lymph node positivity and advance stage. Further, high PLR was significantly associated with lymph node positivity. Regarding tumor associated immune cells, patients with high tumoral and stromal infiltration of CD3+ T cells was associated with histological grade III tumor and advanced stage. Similarly, MPO+ neutrophils infiltration in tumor and stroma tended to be high in patients with premenopausal status and large tumor size. Further, with respect to inflammatory cytokines and hypoxia related markers, IL-8 expression was noted high in patients with young age and larger tumor size. HIF-1 expression was significantly higher in grade III tumors and significant high incidence of tumoral TNF-α expression in tumor was noted in patients with T3 tumor size.
Conclusion: The present study observed lymphopenia and neutrophilia with high NLR, high PLR and its association with disease aggressiveness. Further, infiltration of CD3+ T cells in tumor microenvironment was seen in tumors with advanced histological grade of the tumor, whereas tumor infiltrating neutrophils, TNFα and IL-8 may be cytotoxic to tumor cells via an oxygen radical dependent mechanism.

INTRODUCTION

According to the GLOBOCAN 2018, incidence of female breast cancer is 2.1 million worldwide. [1] Triple negative breast cancer (TNBC) accounts for approximately 15-20% of all breast cancers diagnosed. [2] Similar incidence of TNBC was noted in India as well as in Gujarat Cancer & Research Institute (GCRI). TNBC defined by the absence of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 (Her-2-neu) expression.[2] Recurrence and disease progression are relatively common for women with TNBC, with a peak risk of recurrence within the 1-3 years after diagnosis.[3] A large tumor size, nodal involvement and poor clinical outcomes for women with TNBC may in part be explained by intrinsically aggressive tumor pathology, including high mitotic index, high histological grade, high proliferation and a high frequency of TP53 mutations associated with a frequent occurrence of visceral metastases and poor prognosis.[4]Hypoxia can induce Inflammation which has been shown to be an important factor in the development of tumorigenesis.[5] Various pro-inflammatory cytokines and hypoxic factors produce a systematic inflammatory response which is responsible for the alteration in circulating white blood cells.[7] Hanahan and Weinberg proposed that the tumor microenvironment is infiltrated by innate and adaptive immune system cells specially T lymphocytes that enable tumors to mimic inflammatory conditions seen in normal tissues.[6] Inflammation and hypoxia both conditions play critical role in tumor progression.[9] Therefore, in the present study

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circulating immune cells such as neutrophils, lymphocytes and their ratios such as NLR and PLR and tumor associated immune cells such as CD3+ T cells and MPO+ Neutrophils and hypoxic markers such as IL-8, TNF-α and HIF-1 were evaluated in tumor tissue to investigate their role in tumor development and disease prognosis.

MATERIALS AND METHODS

Study design

Patients: In this retrospective study, 50 female triple negative breast cancer (TNBC) patients enrolled who had been diagnosed and treated at Gujarat Cancer and Research Institute (GCRI) during the period of year 2012 to 2013. The detailed clinical history such as patient's age, menopausal status, disease stage, histopathological findings, hemogram at diagnosis, treatment offered and disease status was recorded from the case files maintained at the Institutional Medical Record Department. Formalin fixed paraffin embedded tissue blocks (FFPE) were retrieved from Histopathology Department for immunohistochemistry. Preoperative differential WBC count of only 34 TNBC patients was available and recorded from haematology department, and 25 healthy female healthy controls were included for comparison. Patients treated with neoadjuvant chemotherapy and stage IV disease were excluded in this study. This study was approved by the Institutional Scientific Review and Ethics Committees.

Immunohistochemical localization: The 4µm thin sections were cut on microtome (Leica, Germany) and taken on 3-aminopropyl triethoxysilane (APES) coated slides. Immunohistochemical localization of CD3+ T cells, MPO+ neutrophils, HIF-1, IL-8 and TNF-α was performed on FFPE tissue blocks containing primary tumor and evaluated by Haematoxylene and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Briefly, the protocol includes following steps of deparaffinization using EZ solution, antigen retrieval using cell conditioning (CC1), incubation with ultra view DAB inhibitor for 4 minutes, 100µl of primary antibody, ultra view HRP multimer for 8 minutes, ultra view DAB detection kit for 8 minutes, counter stain with haematoxylin for 8 minutes, bluing reagent for 4 minutes and mounted with DPX. The primary antibody clone, company, and antibody dilution used are as follows:

Primary antibody	Clone	Company Name	Dilution	Primary antibody incubation time (mins)
CD3	F7.2.38	Dako	1:100	32
MPO	Ab-1	Thermo scientific	1:30	32
HIF-1	H1alpha67	GeneTex	1:30	20
IL-8	807	Abcam	1:50	32
TNF-α	52B83	Abcam	1:200	120

Scoring: CD3+ T cells and MPO+ neutrophils were scored in stroma and stromal infiltration along with tumor core and tumor margin. HIF-1, IL-8 and TNF-α scored as 0 Negative, 1+ (<10% cells stained), 2+ (10-40% cells stained) and 3+ (≥40% cells stained).

Statistical analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Mean, standard deviation and median were calculated and Pearson's chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between the two parameters. The p value ≤0.05 were considered significant.

RESULTS

Leukocyte subset

Neutrophils and lymphocytes count, neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were evaluated in 34 TNBC patients. These parameters were compared with 25 healthy controls.

Comparison of leukocyte subsets and their ratios between TNBC patients and healthy Controls

In comparison to healthy controls, mean percentage of neutrophils (P=0.006) and NLR (P=0.03) was significantly higher, whereas mean percentage of lymphocytes (P=0.002) was significantly lower in TNBC patients. However, a trend of higher PLR was noted in TNBC patients.

Correlation of leukocyte subset and their ratio with clinicopathological parameters

The median value of leukocyte subsets and their ratios was used as a cutoff for correlation with clinicopathological parameters. A trend of higher percentage of neutrophils was noted in patients with T3 tumor size, lymph node positivity, stage III disease, and histological grade I and II tumors. The suppression of lymphocytes was significantly high in patients with lymph node positivity (P=0.02) and a trend was seen in patients with premenopausal status, T3 tumor size, stage III disease, and histological grade I and II tumors. Similarly, a trend of higher NLR was noted in patients with premenopausal status, T3 tumor size, lymph node positivity, stage III disease, and histological grade I and II tumors. The PLR was significantly high in patients with lymph node positivity and a trend was seen in patients with stage III disease (Table 1).

Incidence of tumor infiltrating lymphocytes and their comparison with clinicopathological parameters

In tumor microenvironment, infiltration of CD3+ T cells was noted in 68% (34/50) of TNBC patients. Of them, stromal infiltration was noted in 47% (16/34) patients and infiltration in tumor core and margin along with stroma was noted in 53% (18/34) of TNBC patients (Figure 1). Higher incidence of CD3+ T cells in stroma was observed in patients with histological grade III tumors (P=0.01) and in patients with stage III disease. Furthermore, significantly higher incidence of CD3+ T cells in tumor core and margin along with stroma was observed in patients with histological grade III and BR score 8 tumors and a similar trend was seen in patients with T3 tumor size, lymph node positivity, and stage I disease.

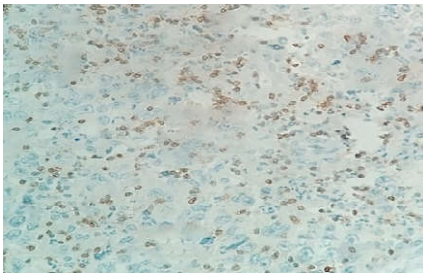

Figure 1 Infiltration of CD3+ T cells in tissue of TNBC patients

Table 1 Correlation of leukocytes subsets and their ratio with Clinicopathological variables in TNBC patients

Parameters		N (%)	Neutrophils Median= 64		Lymphocytes Median=25.6		NLR Median=2.5		PLR Median=11.6	
			Low N (%)	High N (%)	Low N (%)	High N (%)	Low N (%)	Low N (%)	Low N (%)	High N (%)
Age	<50	20(59)	10(50)	10(50)	10(50)	10(50)	11(55)	9(45)	10(50)	10(50)
	≥50	14(41)	8(57)	6(43)	7(50)	7(50)	9(64)	5(36)	7(50)	7(50)
Menopausal Status	Pre menopausal	14(41)	6(43)	8(57)	8(57)	6(43)	6(43)	8(57)	7(50)	7(50)
	Post menopausal	20(59)	12(60)	8(40)	9(45)	11(55)	14(70)	6(30)	10(50)	10(50)
Tumor Size	T1	3(09)	3(100)	0(0)	1(33)	2(67)	2(67)	1(33)	2(67)	1(33)
	T2	26(76)	13(50)	13(50)	13(50)	13(50)	16(61)	10(39)	13(50)	13(50)
	T3	5(15)	2(40)	3(60)	3(60)	2(40)	2(40)	3(60)	2(40)	3(60)
Lymph node Stage	Positive	12(36)	4(33)	8(67)	9(75)	$3(25)_a$	5(42)	7(58)	1(8)	$11(92)_b$
	Negative	21(64)	14(67)	7(34)	7(33)	$14(67)^a$	15(71)	6(29)	16(76)	$5(24)^b$
	I	2(06)	2(100)	0(0)	0(0)	2(100)	2(100)	0(0)	2(100)	0(0)
	II	27(79)	15(56)	12(45)	14(51)	13(49)	16(59)	11(41)	14(52)	13(48)
	III	5(15)	1(20)	4(80)	3(60)	2(40)	2(40)	3(60)	1(20)	4(80)
Histopatho logical Type	Invasive ductal carcinoma	33(97)	18(55)	15(46)	16(49)	17(51)	20(61)	13(39)	17(51)	16(49)
	Medullary Carcinoma	1(03)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
Grade	I	1(04)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
	II	15(53)	7(47)	8(53)	8(53)	7(47)	8(53)	7(47)	7(47)	8(53)
	III	12(43)	8(67)	4(33)	4(33)	8(67)	9(75)	3(25)	7(58)	5(42)
BR score	5	1(04)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
	6	7(25)	3(43)	4(57)	4(57)	3(43)	4(57)	3(43)	3(43)	4(57)
	7	7(25)	3(43)	4(57)	4(57)	3(43)	3(43)	4(57)	4(57)	3(43)
	8	13(46)	9(69)	4(31)	5(39)	8(61)	10(77)	3(23)	7(54)	6(46)

a X2=5.308, r=-0.41 and p=0.021;b: X2=14.078, r=0.653 and p=0.0001

Table 2 Correlation of CD3+ T cells and MPO+ Neutrophils with clinicopathological parameters

Parameters		N	CD3		MPO		HIF-1	IL-8	Tumoral TNFα	Lympho cyticTNFα
			Group-1 N (%)	Group-2 N(%)	Group-1 N (%)	Group-2 N (%)				
Age	<50	29 (58)	8(28)	11(38)	12(41)	5(18)	26(90)	11(38)	21(72)	22(76)
	≥50	21(42)	8(38)	7(33)	8(38)	1(5)	19(91)	13(62)	14(67)	19(91)
Menopausal Status	Pre menopausal	18(36)	4(22)	8(44)	7(39)	5(28)a	16(89)	8(44)	13(72)	14(78)
	Post menopausal	32(64)	12(38)	10(31)	13(41)	1(3)a	29(91)	16(50)	22(69)	27(84)
Tumor Size	T1	3(06)	0(0)	3(100)	2(67)b	1(33)c	3(100)	1(33)	3(100)h	2(67)
	T2	39(78)	15(39)	11(28)	16(41)b	2(5)c	34(87)	18(46)	24(62)h	32(82)
	T3	8(16)	1(12)	4(50)	2(24)b	3(38)c	8(100)	5(62)	8(100)h	7(87)
Lymph node Stage	Positive	28(57)	7(25)	12(43)	13(46)	2(8)	26(93)	14(50)	19(68)	23(82)
	Negative	21(43)	9(43)	5(24)	7(33)	3(15)	18(86)	9(43)	15(71)	18(86)
	I	2(04)	0(0)	2(100)	2(100)	0(0)	2(100)	1(50)	2(100)	2(100)
	II	37(74)	11(30)	13(35)	13(35)	5(14)	33(89)	19(51)	24(65)	31(84)
	III	11(22)	5(46)	3(27)	5(46)	1(8)	10(91)	4(36)	9(82)	8(73)
Histopatho logical type	Invasive Ductal Carcinoma	49(98)	16(33)	17(34)	20(41)	6(12)	44(90)	24(49)	35(71)	40(82)
	Medullay Carcinoma	1(02)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)
Grade	I	2(05)	1(50)d	0(0)e	1(50)	0(0)	1(50)f	2(100)	2(100)	2(100)
	II	26(59)	7(27)d	6(23)e	9(34)	2(8)	24(92)f	11(42)	17(65)	20(77)
	III	16(36)	7(44)d	9(56)e	7(44)	3(19)	16(100)f	8(50)	11(69)	13(82)
BR score	5	4(10)	2(50)	0(0)	2(50)	1(25)g	3(75)	3(75)	3(75)	3(75)
	6	13(30)	5(39)	2(15)	3(23)	1(8)g	12(92)	5(38)	8(62)	10(77)
	7	9(20)	1(11)	3(33)	4(44)	0(0)g	7(78)	5(56)	6(67)	7(78)
	8	15(34)	4(27)	10(67)	8(54)	2(13)g	14(93)	7(47)	11(73)	12(80)
	9	1(03)	1(100)	0(0)	1(100)	0(0)g	1(100)	1(100)	1(100)	1(100)

Group-1 Stromal infiltration
Group-2 Stromal + tumor core and margin infiltration
a: X^2=7.104, r=-0.34 and p=0.029 b: X^2=9.903, r=0.05, p=0.042 c: X^2=9.903, r=0.05, p=0.042 d:X^2=6.04, r=0.143, p=0.049, e: X^2=12.89, r=0.48, p=0.012; f: X^2=12.89, r=0.48, p=0.012; g: X^2=7.07, r=0.318, p=0.029: hX^2=16.64, r=0.46, p=0.034.

Further, the infiltration of MPO+ neutrophils was noted in 52% (26/50) of TNBC patients. Of them, stromal infiltration was noted in 77% (20/26) patients and infiltration in tumor core and margin along with stroma was noted in 23% (6/26) of TNBC patients (Figure 2). A significantly higher incidence of PO expression in stroma was observed in patients T1 tumor size (P=0.042).

However, significantly higher incidence of MPO expression in tumor core and margin along with stroma were observed in patients with premenopausal status and T3 tumor size (P=0.042).

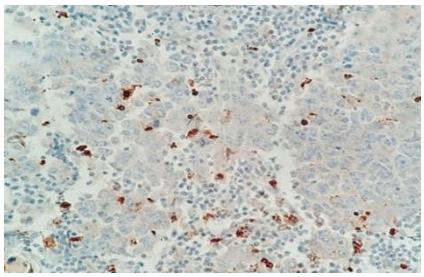

Figure 2 Infiltration of MPO+ neutrophils in tissue of TNBC patients

Regarding Hypoxia and inflammatory markers, expression of HIF-1, IL-8 and TNF-α was seen in tumor and stroma of TNBC patients. Incidence of HIF-1 expression was noted in 90% (45/50) with an intensity of 1+, 2+ and 3+ was noted in 27% (12/45), 38% (17/45) and 35% (16/45) of patients, respectively (Figure 3). A significantly high incidence of HIF-1 expression was noted in patients with histological grade III tumors (p=0.029). Expression of IL-8 was noted in 48% (24/50) with an intensity of 1+ (Figure 4). A trend of high incidence of IL-8 expression was noted in patients with age <50 years and T3 tumor size. Expression of TNF-α in tumor was noted in 70% (35/50) of TNBC patients with an intensity of 1+, 2+ and 3+ was noted in 40% (14/35), 46% (16/35) and 14% (5/35) patients respectively (Figure 5). A significant high incidence of TNF-α expression in tumor was noted in patients with T3 tumor size (P=0.034). Further, expression of TNF-α in lymphocytes was noted in 82% (41/50) of TNBC patients with, an intensity of 1+, 2+ and 3+ in 56% (23/41), 24% (10/41) and 20% (8/41) patients respectively. Trend of high incidence of TNF-α expression was noted in patients with age ≥50 years and stage I disease (Table 2).

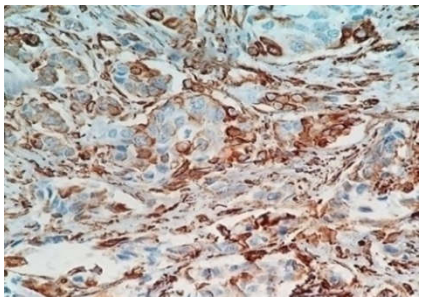

Figure 3 HIF-1 expression in tissue of TNBC patients

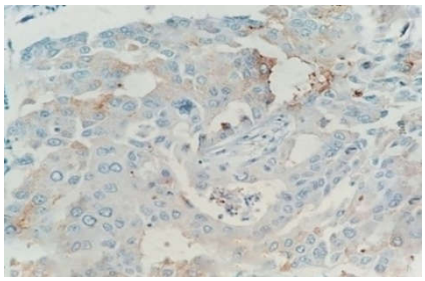

Figure 4 IL8 expression in tissue of TNBC patients

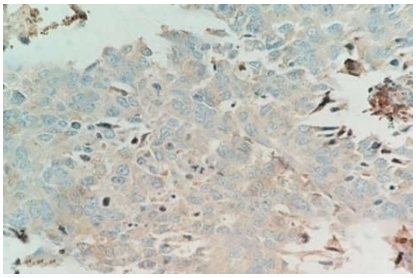

Figure 5 TNFα expression in tissue of TNBC patients

Intermarker correlation

Leukocyte subsets and their ratios were intercorrelated with each other. A significant inverse correlation was found between Neutrophils and Lymphocytes; Lymphocytes and PLR; Lymphocytes and NLR, whereas a positive correlation was found between Neutrophils and NLR; Neutrophils and PLR; NLR and PLR, MPO and tumoral TNF-α; tumoral TNF-α and lymphocytic TNF-α; IL-8 and lymphocytic TNF-α (Table-3).

Table 3 Intermarker correlation

Intermarker correlation of tumor infiltrating markers						
		MPO	**HIF-1**	**Tumoral TNF-α**	**Lymphocytic TNF-α**	**IL-8**
CD3	r	0.19	0.34	0.02	0.12	0.14
	p	0.26	0.05	1.00	0.62	0.47
MPO	r		0.08	0.33	-0.03	0.28
	p		0.92	0.04*	1.00	0.08
HIF-1	r			-0.07	-0.02	0.05
	p			1.00	1.00	1.00
Tumoral TNF-α	r				0.48	0.28
	p				0.01*	0.09
Lymphocytic TNF-α	r					0.34
	p					0.03*

Intermarker correlation of blood subsets and their ratios				
		Lymphocytes	NLR	PLR
Neutrophils	R	-0.707	0.825	0.471
	p	0.0001*	0.0001*	0.006*
Lymphocytes	R		-0.882	-0.647
	p		0.001*	0.001*
NLR	R			0.647
	p			0.0001*

*p value ≤ 0.05 is significant

DISCUSSION

Since long it has been recognized that some tumors are densely infiltrated by cells of both innate and adaptive arms of the immune system and thereby inflammatory conditions arising in non-neoplastic tissues.[10] The pretreatment counts of peripheral inflammatory cells, including neutrophils, lymphocytes and monocytes, have demonstrated the strong link between the inflammatory system and prognosis in different types of cancer.[11]In present study, leukocytes subsets and their ratio were compared with healthy controls. Due to inflammatory response, significantly increased neutrophils count, decreased lymphocytes counts and high NLR were found in TNBC patients in comparison with healthy controls. In relation to clinic pathological variables, increased neutrophils, high NLR and decreased lymphocytes were associated with large tumor size, lymph node positivity and advance disease stage. Further, high PLR was significantly associated with lymph node positivity. In accordance Krenn-Pilko *et al* have shown association of high NLR associated with the presence of a large tumor and a higher T classification, advanced disease, high histological grade in breast cancer patients. [12]

In this study survival analysis was not performed due to small sample size and only 6 patients developed disease relapse. Studies by Azab *et al* and Pistelli *et al* showed increased pretreatment NLR may be associated with worse DFS and OS in patients with early TNBC patients [14, 15]. Adam *et al.* (2015) have stated that tumors are infiltrated by a heterogeneous population of immune cells, such as T-cells, B-cells, natural killer (NK) cells and macrophages.[15] Tumor infiltrating lymphocytes (TILs), a primary immune component infiltrating solid tumors, are considered to be the manifestation

of the host antitumor reaction.[16] The majority of TILs in solid tumors are of the CD3+ T-cell phenotype, which includes CD4+ helper cells (Th1 and Th2 subtypes), CD4+ regulatory T-cells and CD8+ cytotoxic T lymphocytes (CTLs).[17] In the present study, significantly high stromal infiltration CD3+ T cells were noted in patients with histological grade III tumors along with tumor core and margin. In our previous study on Oral squamous cell carcinoma showed that cytotoxic T cells in tumor stroma were significantly low in patients with Stage III disease as compared to Stage I, Stage II, and Stage IV disease. [18] In a multicentric study, Immunoscore (CD 3 and CD8 score) provides a reliable estimate of the risk of recurrence in patients with colon cancer. These results support the implementation of the consensus Immunoscore as a new component of a TNM-Immune classification of cancer. [19] Moreover, tumor cells produce many chemokines that may be varying in different tumor compartments and, therefore, variable densities of tumor infiltrating T cells were observed within different tumor compartments.

Neutrophils secrete MPO and binding of MPO to MMR (Macrophage Mannose Receptor) induces secretion of reactive oxygen intermediates, IL-8, TNF-α and GM-CSF in chronic inflammatory environments.[15] High MPO activity or MPO+ cell infiltration has been detected in esophageal,[20] gynecological,[21] and in colorectal cancers [22] but their prognostic impact was not analyzed. In the present study, it was observed that stromal infiltration of neutrophils was significantly higher in patients with small tumor size; however, stromal infiltration along with tumor core and margin infiltration was significantly higher in patients with larger tumor size. Therefore, MPO expression may be associated with tumor burden.

Hypoxia induces the activity of HIF-1α, downstream signaling activates transcription of erythropoietin, VEGF, glycolytic enzyme coding genes which are implicated in vasodilation, neovascularization, and tumor metastasis.[23]In current study, significant high incidence of HIF-1 expression was noted in patients with histological high-grade tumor. Similar to our findings, Bos *et al*. revealed a positive association between increased proliferation, poor histologic grade and high levels of HIF1α in breast cancer.[24] Our finding suggests that high grade tumors are more hypoxic as compared to low grade tumor.

IL-8 is an important chemo attractant in the context of neutrophil recruitment.[25] Regulation of IL-8 within the tumor microenvironment is complex, not only because of the variety of cells that can secrete it but also because of the multitude of factors that can affect IL-8 expression by different cell types.[26] Various cytokines such as IL-1β, tumor necrosis factor-alpha, IL-6; growth factors such as epidermal growth factor; and hormones such as estrogen and progesterone have shown to up regulate IL-8 expression in breast cancer cells.[27] In the present study, a trend of high incidence of IL-8 expression was noted in patients with younger age and large tumor size may be due to disease aggressiveness. Further, correlation of IL-8 expression with clinicopathological parameters was not reported yet.

Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine involved in apoptosis, inflammation and immunity.[28] The major sources of TNF are macrophages and to a lesser extent T lymphocyte, proliferating B cells, natural killer (NK) cells, mast cells and stimulated neutrophils.[29] In the present study, significant higher incidence of tumoral TNF-α expression in tumor was noted in patients with large tumor size. Regarding survival, Salgado *et al*. (2015) showed that presence of tumor infiltrating lymphocytes in preoperative TNBC patients, have positive correlation with improved overall survival; increased metastasis-free survival.[30],[31] Only one author Wang *et al*. (2016) showed that hepatocellular carcinoma patients with TNF-α have shorter survival time than those with low TNF-α expression.[32]

Regarding intermarker correlation, significant inverse correlation was observed between lymphocytes and neutrophils, NLR and PLR and a significant positive correlation between neutrophils and NLR as well as PLR suggest impaired immune function. Regarding tissue infiltrating cells, positive correlation of neutrophils was observed with tumoural and lymphocytic TNFα and IL8. Hachiya *et al*. (2000) indicated for the first time that irradiation inhibits the expression of MPO mRNA through the autocrine pathway which involves the endogenous production of TNF-α in HL60 cells.[33]Osawa Y*et al*., (2002) demonstrates that TNF-α treatment induces IL-8 mRNA expression in hepatocytes and the production of IL-8 was mediated through NF-kβ and Akt signaling cascades involved in TNF-α induced signaling pathways, and this chemokine exerted antiapoptotic and mitogenic effects on hepatocytes.[34]

In conclusion, the present study observed lymphopenia and neutrophilia with a high NLR, high PLR and their association with disease aggressiveness. Further, infiltration of CD3+ T cells in tumor microenvironment was seen in tumors with advanced histological grade of the tumor, while tumor infiltrating neutrophils, TNFα and IL-8 may be cytotoxic to tumor cells via an oxygen radical dependent mechanism.

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