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Original Article

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Cytoplasmic CCR7 (CCR7c) Immunoexpression Is Associated with Tumor Invasion in Gastric Cancer

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ABSTRACT

Background: This study investigates the CCR7 chemokine receptor's prognostic value in gastric cancer and its relationship to metastasis.

Materials and Methods: Normal and adjacent tumor cells in 70 patients with gastric cancer were evaluated for CCR7 expression using immunohistochemical staining. The prognostic values of high and low levels of expression of CCR7 were also evaluated by multivariate and univariate analyses.

Results: Analysis indicated high expression of CCR7 in 52.9% of tumor tissue. Moreover, high expression of CCR7 was significantly related to metastasis of lymph nodes (p = 0.00). In addition, high expression of CCR7 had a positive correlation to the disease stage (p = 0.00), age of ≥50 years (p = 0.019), male gender (p = 0.024), vascular involvement (p = 0.009), histology of tumor adenocarcinoma (p = 0.00), and poor tumor differentiation (p = 0.00). However, the high expression of the CCR7 marker was not related to the tumor size.

Conclusion: Based on our results, CCR7 expression in gastric cancer can be considered a clinical prognostic indicator in patients with gastric cancer.

Keywords: CCR7; Gastric cancer; Metastasis; Prognosis

INTRODUCTION

Chemokines are among the important members of chemotactic cytokines. These proteins regulate cellular immunity by migrating leukocytes and directing cells' immune response to the infection and inflammation site through lymphatic organs[1]. CCR7 is a common receptor between chemokines CCL19/ELC and CCL21/6Ckine that has essential functions in the immune response of lymphocytes[2]. Endothelial veins, lymph nodes, spleen T-cell zone, peyer's patch, and lymphatic endothelium of many organs have the expression sites of CCL21/6Ckine. Both CCL19/ELC and CCL21/6Ckine are expressed with the highest intensity in lymph nodes. Nevertheless, quantitative analysis has revealed the expression of CCL21/6Ckine in higher density. This finding demonstrates CCR7's vital role in homing lymph node immune cells[3,4]. CCR7 is the receptor of these two ligands in all naive T cells, mature dendritic cells, B cells, and some memory T cells. Accordingly, it has critical functions in lymphocyte homing and trafficking in the lymph nodes[5, 6].

Research has shown the significant role of CCR7 in metastasis and invasion[7]. This receptor induces metastasis in some tumor cells. Tang et al.[8] showed the regulatory role of the Let-7a/CCR7 axis in the

development of epithelial-mesenchymal transition (EMT) in prostate cancer cells. Besides, they showed that this axis activates the P38/ERK signaling pathway to mediate the tumor cells' migration and invasion process. Li et al.[9] attributed the CCR7 marker's high expression to lymph node metastasis. According to these researchers, this marker could independently prognosticate the survival of the patients suffering from Triple-Negative breast cancer (TNBC). In another study, Du et al.[10] introduced the high CCR7 expression levels as a negative predictor of gastric cancer. Up until now, little efforts have been made to examine on the expression of CCR7 in patients with gastric cancer, with contradictory results on the relationship of marker expression to the clinicopathologic factors.

The present study evaluates the relationship between the expression of CCR7 using immunohistochemistry and the prognostic findings in gastric cancer patients.

MATERIALS AND METHODS

Research type and participants

This practical case-control research investigates the expression of CCR7 in the gastric cancer patients' tumor tissue. To this end, the participants were divided into a control group and a case group with the tumor-adjacent normal tissue in the same slide. The research's major objective was to study the association between the expression of the CCR7 marker and the clinicopathologic characteristics in the malignant tumor on the gastric cancer tissue samples from 2017 to 2022. The selected samples were acquired in the archive of the Pathology Department of Imam Khomeini Hospital, Sari, Mazandaran Province (Iran).

The participants were divided into two groups: 1) < 50 years and 2) > 50 years. Moreover, the tumor sizes were classified into two groups, namely <5 cm and ≥5 cm.

Inclusion criteria

The research population was patients diagnosed with gastric cancer after the surgery and those not subjected to chemotherapy prior to surgery.

Exclusion criteria

In this study, we excluded the patients with incomplete clinicopathologic information. In addition, tumors with slides or blocks inappropriate for hematoxylin-eosin or immunohistochemical staining were discarded. Eventually, each study group was assigned with 70 patients**.**

Data collection

The collected samples not subjected to radiotherapy and chemotherapy were acquired from Imam Khomeini Hospital. In the next step, the corresponding checklist was completed.

Afterward, the needed paraffin blocks were removed from the archive. Finally, hematoxylin-eosin-stained slides were prepared from invasive tumoral and normal tissues as controls. These tissues also included apparently tumor-adjacent normal tissues.

Microscopic parameters (e.g., lymphatic invasion, penetration depth, vascular invasion, the presence of tumor, and tumor differentiation level) were also studied.

In the immunohistochemistry approach, first, 4-µm sections of the chosen block were put on Saylyn S3003 slides at 60°C for 1 h. Next, in three steps, they were treated with absolute ethanol, xylol, and ethanol 96% for deparaffinization. The exposure was two times for each solution, each time lasting 5 min. Eventually, they were rinsed with the running water. Following the drying, internal peroxidase was eliminated by transferring the slides to methanol and 1% hydrogen peroxide mixture and placed in the target solution after 10 min. Then, the slides were put in an autoclave for 13 min at 105 MmHg/h20 pressure until reaching the boiling point. In the next step, we declined the microwave power below 40%. Afterward, the tissues were taken out after 15 min and left until reaching room temperature. The tissues were rinsed with wash buffer and tap water. After the rinsing step, a Dako stylus was applied to determine their margins. To this end, the tool was put in a moist chamber in the dark. We used the anti-CCR7-antibody marker (1:1000 dilution) to cover the samples. Then, they were put in the refrigerator for 18 h. This study's negative, positive, and control were peripheral blood smear (PBS), splenic tissue,

and tumor-adjacent normal tissue, respectively. In this research, immune staining specificity was examined by the simultaneous run of both negative and positive controls in each experiment. Next, we placed the samples in an envisioned environment for 1 h at room temperature. Finally, they were rinsed twice with a wash buffer.

The slides were poured with 3,3'-Diaminobenzidine (DAB) solution. When they turned brown after 1-2 min, they were placed in the washing buffer for an additional 2 min. In the next stages, the slides were washed using stained with Mayer's hematoxylin and distilled water. After that, they were rinsed in distilled water again and fixed in xylol. In the end, Entellan was used to mount the slides. Two pathologists experienced in CCR7 marker examined and reported the prepared slides. The CCR7 marker's expression pattern was of the cytoplasmic type. Here, we considered the brown cytoplasm as positive. All slides were examined based on their staining intensity, extent and stained cell percentage. Finally, the results were expressed in a semi-quantitative manner. The CCR7 marker's staining index was estimated through multiplication (staining index). More specifically, it was calculated by multiplying the score of cell staining intensity by the score acquired for stained cell proportion. The cell staining intensity was reported using the following equation:

Negative(0), weak staining(1), moderate staining(2), and strong staining(3) The percentage of stained cells was also reported as follows: None staining 1 (1-25%), 2(25-50%), 3 (50-75%) and 4(>75%). Staining index ≥6 was considered as an expression at high intensity and <6 as an expression at low intensity[4].

It is noteworthy that the CCR7 marker used in this study was supplied by ABCAM Co. (USA).

Statistical analysis

The obtained data were analyzed in terms of descriptive statistics, including mean ± standard deviation (mean ± SD) for age, frequency tables, percentages, and ratios. Since both control and case groups were age-matched, CCR7 expressions were compared by conducting Fisher exact, McNemar, and chi-square ($\chi 2$) tests. Considering the matched age, the variables were assessed using conditional logistic regression. STATA12 software was used for data analysis at a significance level of $P < 0.05$.

RESULTS

Table 1 lists the pathological and clinical findings of 70 patients with gastric cancer(GC) participated in the study. CCR7 expression was evaluated by immunohistochemistry in the gastric cancer specimens. According to Figure 1, CCR7 is diffusely expressed in GC tumor cells' cell membranes and cytoplasm.

Strong expression was seen in 14 (20%) patients. In addition, moderate Figure 2 and weak expression Figure 3 was observed in 23 (32.9%) and 20 (28.6%) patients, respectively. Finally, no expression Figure 4 was observed in 13 (18.6%) patients (Table 2).

The marker's positive expression rate in normal tissue adjacent to the tumor was 10 (14.3%). Normal tumor margin tissue and tumor tissue were considered the control and case group, respectively. Table 3 shows the multivariate and univariate analyses of the relationship between clinicopathologic findings and CCR7 expression.

The research participants included 29 (41.4%) female and 41 (58.6%) male GC patients. In this study, 25 (35.7%) patients were below 50 years old, and the others were ≥50 years. Tumor size was <5 cm in 29 (41.4%) patients and ≥5 cm in the others. In addition, 39 (55.7%) patients had vascular involvement, 49 (70%) patients had lymph node metastasis, and 14 (20%) had poorly differentiated tumors.

High expression of CCR7 showed a significant relationship with the male gender ($p = 0.024$), age of >50 years ($p = 0.019$), type of adenocarcinoma histology to diffusion ($p = 0.00$), vascular involvement ($p = 0.009$), disease stage ($p = 0.00$), lymph node metastasis ($p = 0.00$), and tumor differentiation ($p = 0.007$).

Table 1: Clinicopathologic findings of patients with gastric carcinoma

		Number	Percentage
Age(58.4±12.5)	<50 years	25	35.7%
	≥50 years	45	64.3%
Sex	Male	41	58.6%
	Female	29	41.4%
Tumor size(5.42±2.4)	<5 cm	29	41.4%
	≥5 cm	41	58.6%
Histologic type	Adenocarcinoma	60	85.7%
	Diffuse	10	14.3%
Vascular invasion	Yes	39	55.7%
	No	31	44.3%
Lymghnode metastasis	Yes	49	70.0%
	No	21	30.0%
Stage of disease	Ia	4	5.7%
	II a	9	12.9%
	IIIa	11	15.7%
	I b	8	11.4%
	II b	3	4.3%
	IIIb	10	14.3%
	IV	2	2.9%
	II	21	30.0%
	IIC	2	2.9%
Diffenetation of tumor	Poor	14	20.0%
	Moderate	24	34.3%
	Well	32	45.7%

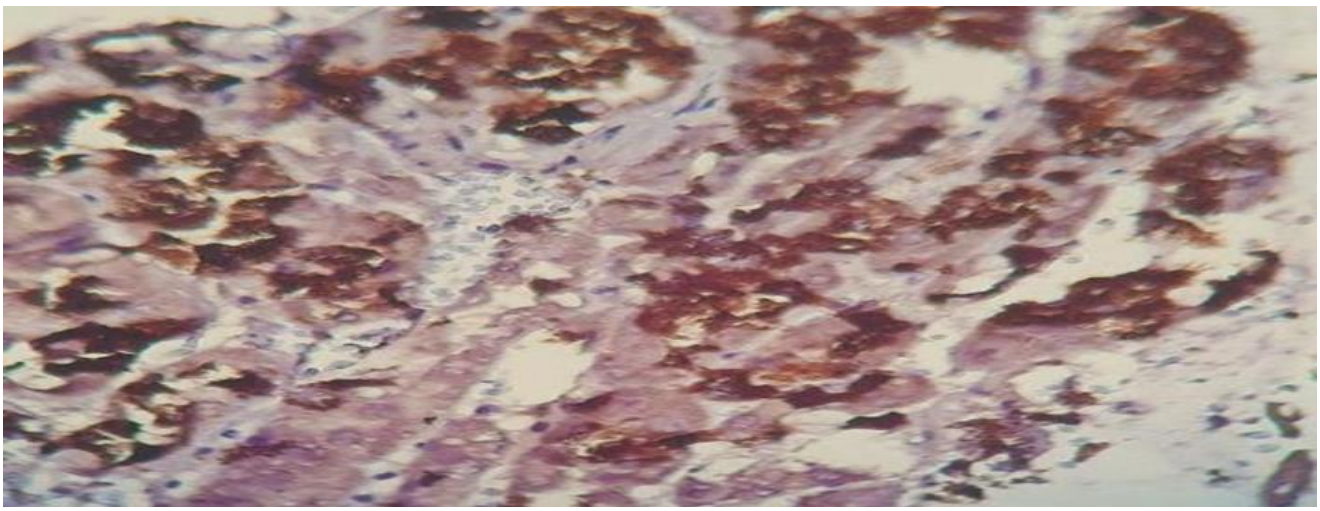

Figure 1. Strong staining of CCR7 marker in the cytoplasm and membrane of gastric tumor tissue cells in immunohistochemical staining (100×)

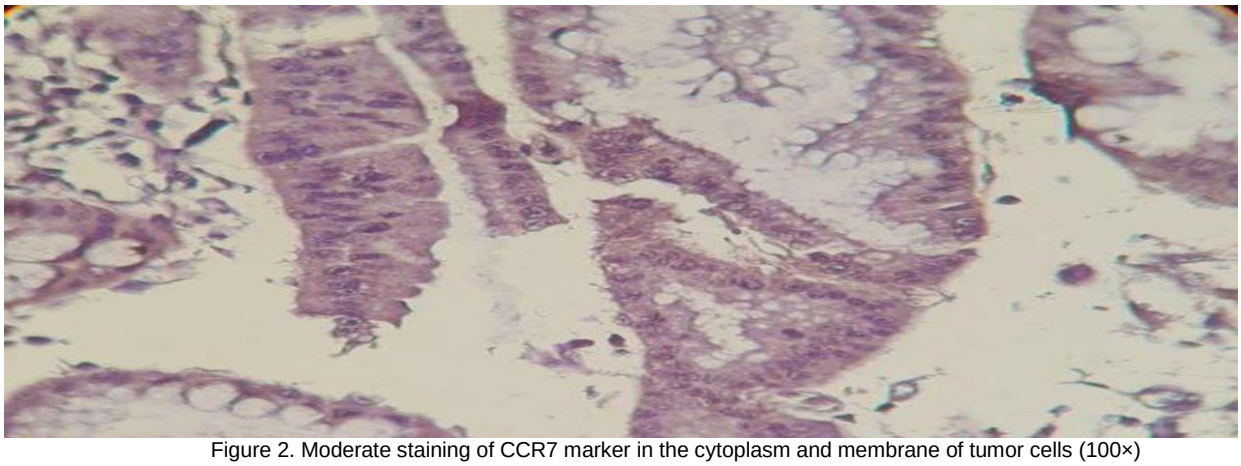

Figure 2. Moderate staining of CCR7 marker in the cytoplasm and membrane of tumor cells (100×)

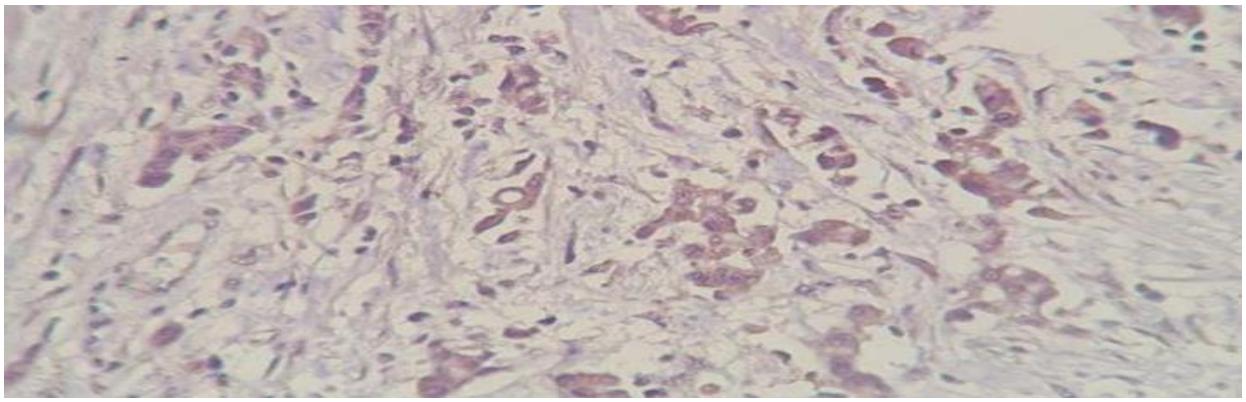

Figure 3. Weak staining of CCR7 marker in cytoplasm and tumor cell membrane in gastric carcinoma in immunohistochemical staining (100×)

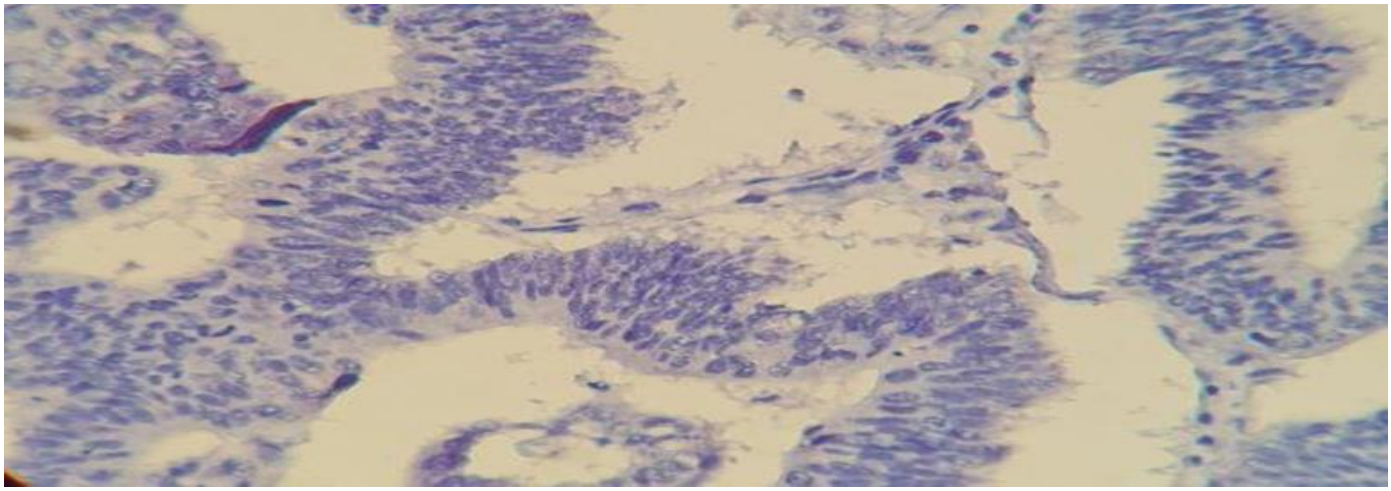

Figure 4. Negative staining of CCR7 marker in cytoplasm and tumor cell membrane in gastric carcinoma in immunohistochemical staining (100×)

Table 2: Comparison of staining intensity of CCR7 marker in the two case and control group

	case		control		p
	Number	Percentage	Number	Percentage	
Negative	13	18.6%	54	77.1%	.000
Weak	20	28.6%	6	8.6%	
Moderate	23	32.9%	7	10.0%	
Strong	14	20.0%	3	4.3%	
Total	70	100.0%	70	100.0%	

Table 3: Relationship between CCR7 expression and clinicopathologic characteristics of gastric carcinoma

Clinicopathologic parameters		case							control						
		Negative		Positive		P	Odds ratio	CI (95%)	Negative		Positive		P	Odds ratio	CI (95%)
		N	%	N	%				N	%	N	%			
Age	<50 years	1	7.7%	24	42.1%	.019	.115	(.014,.942)	11	20.4%	14	87.5%	.000	.037	(.007,.942)
	>50 years	12	92.3%	33	57.9%				43	79.6%	2	12.5%			
Sex	Male	4	30.8%	37	64.9%	.024	.240	(.066,.879)	27	50.0%	14	87.5%	.009	.143	(.03,.690)
	Female	9	69.2%	20	35.1%				27	50.0%	2	12.5%			
Tumor size	<5 cm	3	23.1%	26	45.6%	.137	.358	(.089,1.43)	22	40.7%	7	43.8%	.830	.884	(.286,2.728)
	≥5 cm	10	76.9%	31	54.4%				32	59.3%	9	56.3%			
Histologic type	Adenocarcinoma	5	38.5%	55	96.5%	.000	.023	(.004,.137)	46	85.2%	14	87.5%	.816	.821	(.156,4.324)
	Diffuse	8	61.5%	2	3.5%				8	14.8%	2	12.5%			
Vascular invasion	Yes	3	23.1%	36	63.2%	.009	.175	(.043,.708)	26	48.1%	13	81.3%	.019	.214	(.055,.838)
	No	10	76.9%	21	36.8%				28	51.9%	3	18.8%			
Lymph node metastasis	Yes	2	14.4%	47	82.5%	.000	.039	(.007,.202	34	63.0%	15	93.8%	.018	.113	(.014,.924)
	No	11	84.6%	10	17.5%				20	37.0%	1	6.3%			
Stage of disease	Ia	4	30.8%	0	.0%	.000	9.702	(2.25,41.73)	4	7.4%	0	.0%	.000	2.317	(1.44, 3.72)
	II a	6	46.2%	3	5.3%				9	16.7%	0	.0%			
	IIIa	3	23.1%	8	14.0%				11	20.4%	0	.0%			
	I b	0	.0%	8	14.0%				8	14.8%	0	.0%			
	II b	0	.0%	3	5.3%				3	5.6%	0	.0%			
	IIIb	0	.0%	10	17.5%				7	13.0%	3	18.8%			
	IV	0	.0%	2	3.5%				0	.0%	2	12.5%			
	II	0	.0%	21	36.8%				12	22.2%	9	56.3%			
	IIc	0	.0%	2	3.5%				0	.0%	2	12.5%			
Diffenetation of tumor	Poor	1	7.7%	13	22.8%	.007	.226	(.067,.762)	3	5.6%	11	68.8%	.000	.111	(.038,.327)
	Moderate	1	7.7%	23	40.4%				21	38.9%	3	18.8%			
	Well	11	84.6%	21	36.8%				30	55.6%	2	12.5%			

DISCUSSION

The present study evaluated the association between the expression of CCR7 and the clinical findings and prognostic factors in gastric cancer (GC) patients. In this experimental study, 70 GC patients were examined. Here, the CCR7 marker was introduced as a negative prognostic factor to correlate the high expression of CCR7 with deep tumor invasion (i.e., advanced stages), vascular invasion, lymph node metastasis, the type of weaker differentiation, and histology of adenocarcinoma to the diffusion type. Although there was no significant correlation between CCR7 expression and tumor size, this expression was associated with other clinical variables, including age >50 years and male gender. Reviewing the literature indicates conflicting results regarding the CCR7 prognostic value in GC. For instance, Yan et al.[11] stated that a high CCR7

expression is related to the tumor size; the higher the tumor size, the greater the expression of CCR7. In addition, Wang et al[12]. confirmed the relationship between CCR7 and tumor size >5 cm. However, Yan[11] and Mashino[2] and Zhang[13] found no meaningful association between CCR7 expression and tumor size. Consistent with Zhang et al.'s finding, the present research showed that tumor size was not related to CCR7 expression.

According to Kwack et al.[14], differentiated tumors showed higher CCR7 expression than undifferentiated ones. In addition, Arigami[15] demonstrated that the degree of differentiation and histological findings did not affect CCR7. The present study showed a high expression of CCR7 in poor cases and adenocarcinoma to the diffuse type.

This study's major objective was to examine the association between metastasis and a high CCR7 expression. In this regard, we observed higher CCR7 expression levels in patients with metastasis of lymph nodes. In addition, expression of CCR7 in tumor cell types was correlated to increased metastasis, as confirmed in breast cancer[4] and colorectal cancer[16]. Wang et al.[12] revealed that expression of the CCR7 marker is more common in patients suffering from metastasis of lymph nodes[17], which is also confirmed by Zhuo. In other efforts, Du[10] and Kwack[14] showed a significant correlation between high expression of CCR7 and metastasis of lymph nodes and vascular invasion. However, Mashino[2], Arigami[15], and Ishigan[18] observed a relationship between positive and strong expression of CCR7 and metastasis but not with vascular invasion. A recent study showed that CCR7 is highly activated during GC metastasis. Therefore, this outcome may explain some of the above aggressive traits in this type of cancer. Overall, this finding can be used in treating GC by improving the therapeutic outcome of these patients by inhibiting such receptors.

According to Ma et al.[19], the high CCR7 expression in the GC tumor cells was not related to age and gender. Moreover, Zhuo et al.[17] did not detect any relationship between strong CCR7 expression and age of >60 years and gender. These results are consistent with those reported by Wang and Yan[11, 12]. According to these authors, the high expression of CCR7 is a function of gender (male) and age (>50 years). Yan[11] reported the tumor cells' CCR7 expression level to be 52.9%, which is 53.4% in tumor cells in the present study. The CCR7 expression level in gastric tumor cells was also reported to be 69.9% in Zhuo's[17] study.

Expression of CCR7 was considerably higher in patients at the disease stages VI and III than in the lower stages. This finding was also reported by Zhuo et al[17]. Furthermore, Yan[11], Wang[12], and Zhuo[17] confirmed this finding. Consistent with the above studies, the present research revealed the high CCR7 expression in GC tumor cells was related to a higher clinical stage in patients.

CONCLUSION

Some biological characteristics such as the age of >50 years, male gender, positive lymph node state, poorer degree of differentiation, higher stage of disease, vascular involvement, and biomarkers such as CCR7 can be used to predict whether a GC patient is prone to develop metastatic disease shortly after the initial diagnosis of GC.

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Conflict of Interest

None declared.

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