Neurology Asia 2025; 30(4) : 1217 – 1225

Serum chemokine profiling in spinal ependymoma: Correlation with tumor characteristics and prognostic implications

[1]Noor Saadoon Abdulazeez Alzori *MSC*, [2]Maryam Azimzadeh *PhD*, [3,4]Majid Reza Farrokhi *MD*, [5]Sina Vakili *PhD*, [3]Morteza Jafarinia *PhD*

[1]Department of Biochemistry, Shiraz Branch, Islamic Azad University, Shiraz, Iran; [2]Department of Medical Laboratory Sciences, Khomein University of Medical Sciences, Khomein, Iran; [3]Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; [4]Department of Neurosurgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; [5]Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Spinal ependymomas are rare neoplasms that originate from the ependymal cells lining the central canal of the spinal cord. Since serum levels of cytokines and chemokines are imbalanced in tumorigenesis, this study investigated the serum levels of IL-8, CCL2, and CXCL12 chemokines in patients with spinal cord ependymoma and their correlation with multiple tumor parameters. *Methods:* In the current investigation, blood samples were obtained from 50 patients with spinal ependymomas and 25 healthy individuals, who acted as controls, to assess the levels of specific chemokines. The serum levels of interleukin (IL)-8, chemokine (C-C motif) ligand 2 (CCL2), and C-X-C motif chemokine ligand 12 (CXCL12) chemokines were measured using the enzyme-linked immunosorbent assay (ELISA). In contrast, their gene expression levels were assessed using real-time polymerase chain reaction (RT-PCR). Then, the correlation of these factors with tumor size, grade, recurrence, and Karnofsky Performance Status (KPS) score was investigated. *Results:* The current study demonstrated that serum expression levels of IL-8, CCL2, and CXCL12 were significantly elevated in patients with spinal ependymoma compared to the control group. The cytokine IL-8 showed a significant relationship with tumor size, grade, and KPS score. CXCL12 and CCL2 also had a significant relationship with tumor size and grade.
Conclusion: Based on the current study, IL-8, CXCL12, and CCL2 are significantly elevated in spinal ependymoma patients and linked to tumor progression. These findings underscore the need for future studies to broaden the characterization of spinal ependymoma and develop blood-based markers for these tumors to guide clinical management.

Keywords: Cytokine, chemokine, spinal cord ependymoma, prognosis, diagnosis.

INTRODUCTION

Ependymomas are one of the rare primary neoplasms (3-5%) in children (< 10 years old) and adults (45-55 years old) in the central nervous system (CNS), arising from ependymal cells in the spinal cord.[1] Common symptoms include weakness, back pain, tingling, numbness, and paralysis.[2] Due to diverse histopathological features and clinical presentations, ependymoma diagnosis is challenging. Although treatment options include surgery, radiation, and chemotherapy, the disease recurrence rates are high, and the long-term prognosis is poor.[3] Accordingly, for early diagnosis and treatment monitoring, identifying molecular biomarkers from non-invasive liquid biopsies (e.g., serum and plasma) offers promising alternatives to tissue biopsies for biomarker discovery.

Chemokines are fundamental small cytokines (8-12 kDa) involved in immune responses, inflammation, tumorigenesis, and cancer progression.[4] IL-8 is a multifunctional chemokine produced by various cell types (e.g., monocytes,

Address correspondence to: Morteza Jafarinia, PhD, Shiraz Neurosciences Research Center, Shiraz University of Medical Sciences, Chamran Hospital, Shiraz, 719481564 Iran. Tel: +98-71-36234508, Email: jafarinia@sums.ac.ir

Date of Submission: 16 March 2025; Date of Acceptance: 29 June 2025
https://doi.org/10.54029/2025ukx

neutrophils, and tumor cells) in response to inflammatory stimuli and plays a vital role in inflammation by attracting T cells to damaged sites through neutrophil activation.[5] Current evidence suggests that IL-8 is a crucial factor in neovascularization and tumorigenesis associated with squamous cell carcinoma and other cancers.[6] For example, it was noted that elevated IL-8 serum levels were present at all stages of colorectal and glioma cancers.[7,8] Chemokine (C-C motif) ligand 2 (CCL2), monocyte chemoattractant protein-1 (MCP-1), is one of the first identified chemokines involved in attracting monocytes and macrophages, and its overexpression is closely linked to tumor metastasis, invasion, and immune resistance.[9] Notably, CCL2 has a crucial impact on regulating tumor behavior and enhancing the function of tumor-associated macrophages, leading to the release of cell death-related molecules, a process activated by platelets. However, these macrophages may also facilitate epithelial-mesenchymal transition, making the tumor more prone to metastasis.[10] Stromal cell-derived factor 1 (SDF-1), also known as C-X-C motif chemokine ligand 12 (CXCL12), is a homeostatic CXC chemokine. It was suggested that CXCL12 and its receptors (e.g., CXCR4 and CXCR7) are essential in ovarian epithelial tumor development and metastasis.[11] Furthermore, an elevated level of CXCL12 was reported in bladder, gastric, liver, prostate, and lung cancers.[12]

Despite recent research exploring potential chemokine biomarkers in the serum of spinal ependymoma and medulloblastoma patients, there has been no study focused on chemokine biomarkers specifically in spinal ependymoma patients. Taken together, the current investigation aimed to evaluate the serum levels of IL-8, CCL2, and CXCL12 chemokines in spinal cord ependymoma patients and their relationship with tumor size, tumor grade, tumor recurrence, and Karnofsky Performance Status (KPS) score.

METHODS

Study design and subjects

The current case-control study involved 50 patients diagnosed with spinal ependymoma and 25 healthy participants. Following the approval from the research ethics committees of Islamic Azad University-Arsanjan Branch (IR.IAU.A.REC.1403.025) and patient consent, demographic data and blood samples were collected from all participants.

RNA extraction, cDNA synthesis, and Real-Time PCR analysis

Whole blood (3-5 mL) was collected from participants in ethylenediamine tetraacetic acid (EDTA) tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque density gradient centrifugation. PBMC total RNAs were extracted with TRIzol reagent (Invitrogen, Massachusetts, USA) according to the manufacturer's instructions. RNA purity and concentration were determined using a NanoDrop spectrophotometer, and samples with an A260/A280 ratio of 1.8 to 2.1 were processed for further analysis.

One µg of RNA was converted to cDNA using Takara PrimeScript RT Master Mix (Takara Bio Inc., Japan). SYBR Green chemistry was used for quantitative PCR on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). Custom primers for IL-8, CXCL12, CCL2, and the housekeeping gene GAPDH were designed using Primer3 software and were synthesized by Integrated DNA Technologies. A reaction mixture consisting of 2 µL cDNA, 10 µL SYBR Green Master Mix, 1 µL forward and reverse primers (10 µM each), and 6 µL nuclease-free water was used for each 20 µL reaction. Thermal cycling parameters were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation (15 sec at 95°C) and annealing/extension at 72°C for 1 min. Melt-curve analysis verified the specificity of amplification. Quantification was performed using the $2^{-\Delta\Delta Ct}$ method, and IL-8, CXCL12, and CCL2 levels were normalized to GAPDH.

Chemokine quantification

Peripheral venous blood samples (3–5 mL) from spinal ependymoma patients and healthy controls were collected in serum separator tubes (no anticoagulant). Samples were allowed to clot at room temperature for 30–60 minutes. The serum was separated by centrifugation at 2400 × g for 10 minutes and stored at −80°C until analysis. Levels of IL-8, CXCL12, and CCL2 were assessed using an enzyme-linked immunosorbent assay (ELISA) with commercially available kits (R&D Systems, USA). All assays were carried out according to the protocols provided by the manufacturer.

Statistical analysis

The Kolmogorov–Smirnov Z test was performed to assess the normality of the IL-8, CXCL12,

and CCL2 levels. Data are presented as mean ± standard deviation (SD) or frequency (percent). Logistic regression models, adjusted for potential confounders, were used to assess relationships between chemokine levels and other parameters. To control for multiple comparisons, p-values were adjusted using the Benjamini-Hochberg procedure to control the False Discovery Rate (FDR). Adjusted p-values (q-values) were considered statistically significant at $q < 0.05$. Statistical analyses were conducted using IBM SPSS Statistics v24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Demographic characterization

Table 1 presents the characteristics of the study participants. This study examined 50 patients with spinal ependymoma alongside 25 healthy individuals serving as the control group. This control group comprised 25 participants, including 13 men and 12 women, with an average age of approximately 50 years. The 50 patients with spinal ependymoma consisted of 25 men and 25 women, with an average age of approximately 49 years. Among these patients, 6 had tumors smaller than 3 cm, 30 had tumors ranging from 3 to 5 cm, and 14 had tumors ≥ 5 cm. In terms of tumor grade, 9 patients were classified as grade I, 22 as grade II, and 19 as grade III. Furthermore, regarding disease recurrence, 22 patients experienced recurrence, while 28 did not.

Expression levels of IL-8, CXCL12, and CCL2 in spinal ependymoma patients vs. healthy subjects

In this study, we analyzed the expression levels of the chemokines IL-8, CXCL12, and CCL2 in spinal ependymoma patients compared to healthy controls. The results showed a substantial increase in the three chemokines in patients compared with healthy subjects. IL-8 levels were significantly elevated, indicating increased neutrophil recruitment and angiogenesis in the tumor microenvironment. We observed an increase in the expression of CXCL12, which may promote tumor cell proliferation and immune cell infiltration. The levels of CCL2 were also increased, reflecting monocyte/macrophage activation and proinflammatory signaling (Figure 1). These results reveal a strong chemokine-driven inflammatory environment in spinal ependymoma that may mediate tumor progression and microenvironment remodeling.

Serum IL-8 level in spinal ependymoma patients vs. healthy subjects and correlation with recurrence, tumor size, grade, and KPS score

The serum level of the cytokine IL-8 in patients with spinal ependymoma was significantly higher compared to the control group ($q = 0.0015$) (Figure 2A). Furthermore, there was no significant difference in IL-8 levels between patients who experienced recurrence and those who did not ($q = 0.4222$) (Figure 2B). In contrast, the findings regarding the relationship between serum IL-8 levels and tumor size and grade indicated a significant positive correlation; as tumor size ($q = 0.0015$) (Figure 2C) and grade ($q = 0.0015$) (Figure 2D) increased, the serum IL-8 levels also increased significantly. Additionally, the results related to the KPS index in Figure 2E revealed a significant negative correlation, suggesting that higher serum IL-8 levels are associated with a decrease in the KPS index ($q = 0.0078$).

Table 1: Baseline characteristics of study participants

Characteristics		Ependymoma patients	Healthy control
N		50	25
Male/Female		25/25	13/12
Age		49.98±8.89	50.4±12.4
Tumor size (cm)	<3	6 (12%)	-
	3-5	30 (60%)	-
	>5	14 (28%)	-
Tumor grade	I	9 (18%)	-
	II	22 (44%)	-
	III	19 (38%)	-
Recurrence	Yes	22 (44%)	-
	NO	28 (56%)	-

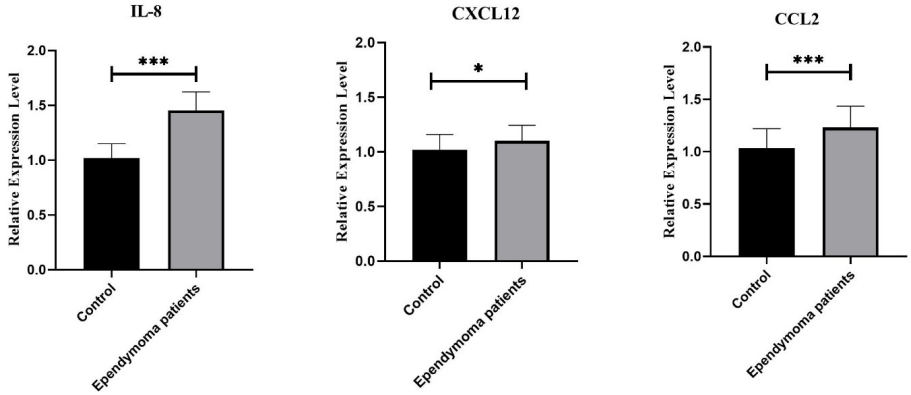

Figure 1. Expression levels of IL-8, CXCL12, and CCL2 in spinal ependymoma patients compared to healthy subjects. The expression levels of IL-8, CXCL12, and CCL2 are displayed in the figure for spinal ependymoma patients versus healthy controls. All three chemokines were significantly higher in patients than in healthy subjects. The results are expressed as the mean ± standard deviation (SD). Statistical significance is indicated as *** for $q < 0.001$, ** for $q < 0.01$, and * for $q < 0.05$, where q-values represent p-values adjusted using the Benjamini-Hochberg method to control the false discovery rate.

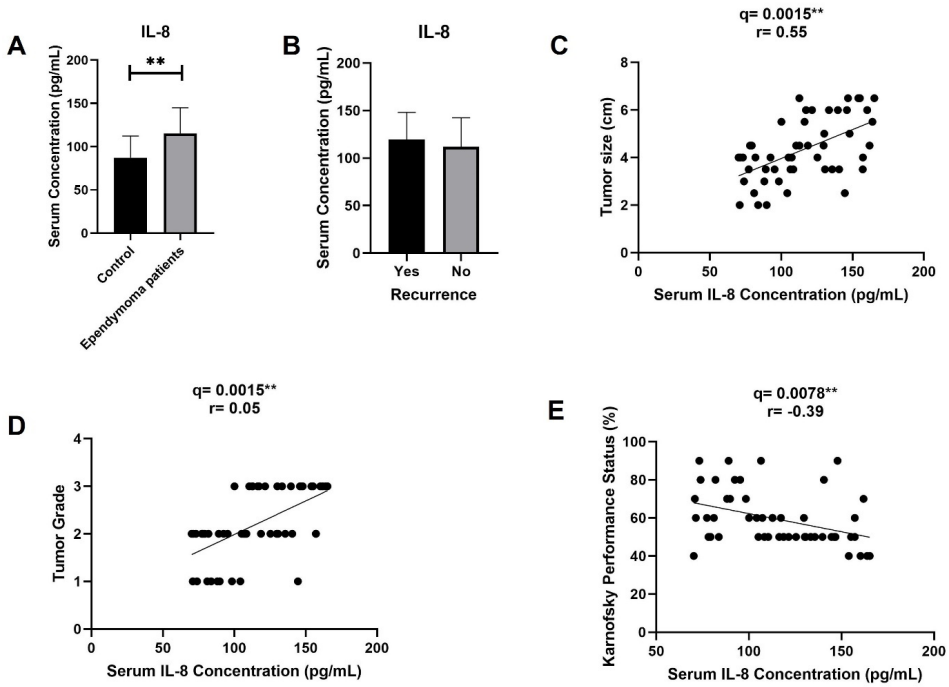

Figure 2. Serum IL-8 levels in spinal ependymoma patients compared to healthy subjects and their correlation with recurrence, tumor size, grade, and KPS score. (A) Serum IL-8 levels in patients with spinal ependymoma were significantly higher than those in the control group. (B) Patients with recurrence exhibited similar levels of IL-8 cytokine as those without recurrence. (C) A strong positive correlation was found between serum IL-8 concentration and tumor size, with serum IL-8 levels significantly increasing alongside tumor size. (D) This strong positive correlation was also noted between serum IL-8 levels and tumor grade, demonstrating significantly increased serum IL-8 levels with each increase in tumor grade. (E) A considerable negative correlation existed between serum IL-8 levels and the KPS index, indicating that heightened serum IL-8 levels correlate with a significant reduction in the KPS index.
Statistical significance is indicated as *** for $q < 0.001$, ** for $q < 0.01$, and * for $q < 0.05$, where q-values represent p-values adjusted using the Benjamini-Hochberg method to control the false discovery rate.
IL-8; interleukin-8, KPS; Karnofsky Performance Status.

Serum CXCL12 level in spinal ependymoma patients vs. healthy subjects and correlation with recurrence, tumor size, grade, and KPS score

The serum CXCL12 level in ependymoma patients was significantly elevated compared to the control group (q = 0.0041) (Figure 3A). Furthermore, as presented in Figure 3B, there was no significant difference in serum CXCL12 levels between patients with recurrence and those without (q = 0.3788). Conversely, the findings related to the correlation between serum CXCL12 levels and tumor size (Figure 3C) and grade (Figure 3D) revealed a significant positive relationship, indicating that as tumor size (q = 0.0036) and grade (q = 0.0109) increase, serum CXCL12 levels are also significantly elevated. Lastly, the results in Figure 3E indicated no significant correlation between serum CXCL12 levels and KPS index (q = 0.4726).

Serum CCL2 level in spinal ependymoma patients vs. healthy subjects and correlation with recurrence, tumor size, grade, and KPS score

The serum level of the CCL2 chemokine in ependymoma patients was significantly elevated when compared to the control group (q = 0.0015) (Figure 4A). In addition, the results presented in Figure 4B show no significant difference in serum CCL2 levels between patients with recurrence and those without (q = 0.4326). The correlation between serum CCL2 levels and tumor size and grade is reported in Figures 4C and 4D, respectively, which indicate that as tumor size (q = 0.0015) and grade (q = 0.0015) increase, the serum CCL2 levels are also significantly elevated. Lastly, the findings in Figure 4E revealed no significant correlation between serum CCL2 levels and the KPS index (q = 0.2320).

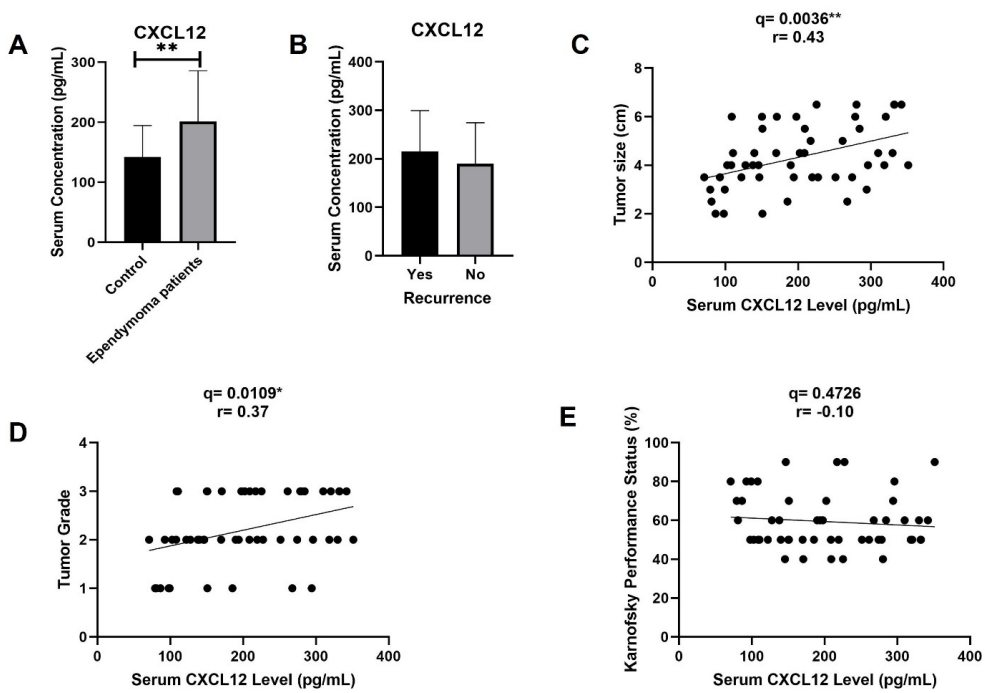

Figure 3. Serum CXCL12 levels in spinal ependymoma patients compared to healthy subjects and their correlation with recurrence, tumor size, grade, and KPS score. (A) Serum CXCL12 levels were significantly higher in ependymoma patients than in controls. (B) There was no significant difference in serum CXCL12 levels between patients who recurred and those who did not. (C) A strong positive correlation was observed between serum CXCL12 levels and tumor size, indicating that higher serum CXCL12 levels correspond to larger tumor sizes. (D) A very strong positive correlation exists between serum CXCL12 levels and tumor grade, suggesting that serum CXCL12 levels are significantly higher in higher-grade tumors. (E) No significant correlation was found between elevated serum CXCL12 levels and KPS index.
Statistical significance is indicated as *** for q < 0.001, ** for q < 0.01, and * for q < 0.05, where q-values represent p-values adjusted using the Benjamini-Hochberg method to control the false discovery rate.
KPS; Karnofsky Performance Status.

DISCUSSION

The findings of the current study indicated that serum IL-8 level was significantly increased in patients with spinal ependymoma, and its overactivity facilitates the tumor growth, angiogenesis, and metastasis.[13] Notably, silencing IL-8 expression results in a decrease in pro-angiogenic factors within the tumor microenvironment, leading to reduced angiogenesis.[14] Furthermore, the current results indicated a significant correlation between increased IL-8 and tumor size and grade. In this context, Najdaghi *et al*. reported that serum IL-8 levels were present at all stages of colorectal cancer (e.g., proliferation, angiogenesis, and metastasis).[7] Fousek *et al*. showed the involvement of IL-8 in various tumorigenesis processes, including the stimulation of tumor cell proliferation, migration, and angiogenesis, along with the modulation of immune response.[6] In another study, Guequén *et al*. noted that IL-8 levels were elevated in glioblastoma, as it is released by glioma cells and regulates the function of the endothelial barrier, promoting tumor progression. IL-8 plays a fundamental role in cancer progression and metastasis through various mechanisms, including pro-angiogenesis and the maintenance of cancer stem cells. Establishing an immune microenvironment could inhibit anti-tumor immune responses.[15] However, its capacity to modulate the functions of immune cells is undoubtedly one of the most important factors.

As the findings of the current study revealed significantly higher levels of CXCL12 and CCL2 chemokines in patients with spinal ependymoma compared to controls, previous related research indicated that CXCL12 and its receptors (e.g., CXCR4 and CXCR7) could play vital roles in cancer progression, angiogenesis, and

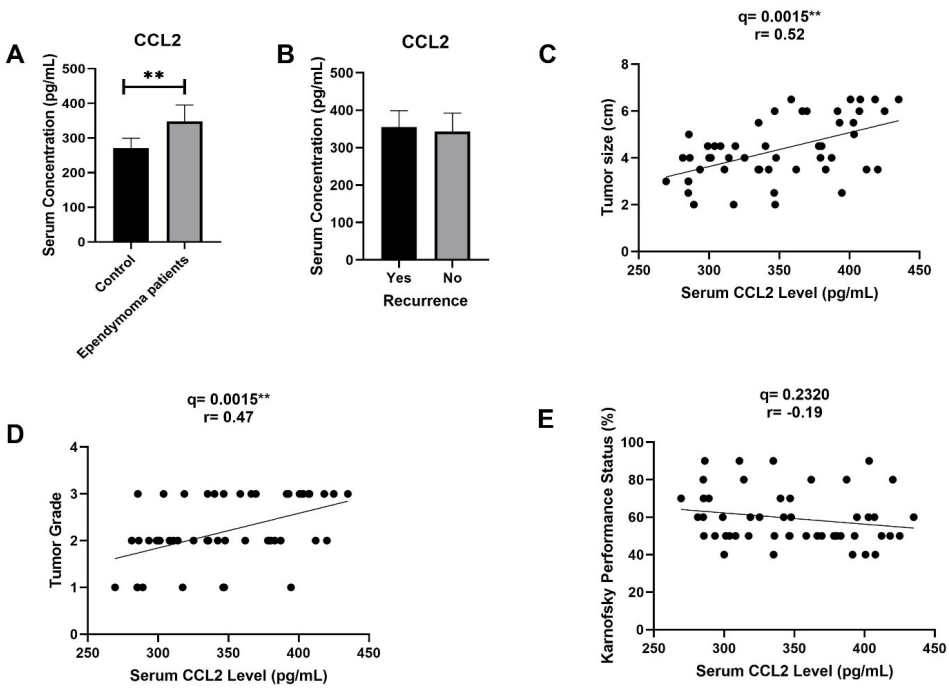

Figure 4. Serum CCL2 levels in spinal ependymoma patients compared to healthy subjects and their correlation with recurrence, tumor size, grade, and KPS score. (A) Serum CCL2 chemokine levels were significantly higher in ependymoma patients than in controls. (B) No differences in serum CCL2 levels were observed between patients with recurrence and those without. (C) A positive correlation was found between serum CCL2 levels and tumor size, indicating that as tumor size increased, serum CCL2 levels also significantly increased. (D) Furthermore, a correlation between serum CCL2 levels and tumor grade was reported, showing that as tumor grade elevates, serum CCL2 levels also rise significantly. (E) There was no significant relationship between serum CCL2 elevation and the KPS index.
Statistical significance is indicated as *** for $q < 0.001$, ** for $q < 0.01$, and * for $q < 0.05$, where q-values represent p-values adjusted using the Benjamini-Hochberg method to control the false discovery rate.
KPS; Karnofsky Performance Status.

chemotherapy resistance.[11] Notably, CXCL12 affects tumor biology through two mechanisms: direct activation of signaling pathways that promote cancer growth and indirect effects involving the localization of its receptors and increased uptake by cancer cells. As a result, the elevated CXCL12 levels are associated with increased tumor cell proliferation.[16] A study conducted by Roberto *et al*. highlighted that the CXCL12 level was elevated in pancreatic adenocarcinoma, which eventually led to tumor growth associated with lymph node metastasis. Accordingly, the CXCL12/CXCR4/CXCR7 signaling axis was crucial for pancreatic cancer development, with variations in receptor expression and CXCL12 binding affecting cancer behavior.[17]

CCL2 is primarily produced by monocytes, but tumor cells can also synthesize it. Its expression is influenced by various transcription factors and is stimulated by factors like tumor necrosis factor (TNF)-α and IL-1β.[18] High CCL2 levels are linked to tumor metastasis, invasion, and immune resistance, although some studies suggest it may help recruit anti-tumor inflammatory monocytes.[19] Pro-inflammatory factors and oncogenes, such as p53, can enhance CCL2 expression. Additionally, tumor-associated macrophages, fibroblasts, and adipocytes contribute to CCL2 production.[20] On the other hand, the CCL2/CCR2 axis plays a crucial role in tumor fibrosis and angiogenesis.[21] Various studies have corroborated the elevated CCL2 levels in tumors. For example, the interaction between CCL2 and IL-33 was critical for esophageal cancer progression. In ovarian cancer, CCL2 released from adipocytes promoted metastasis via the PI3K/AKT/mTOR pathway.[22] Furthermore, breast neoplasms secreted higher levels of CCL2 and IL-8, which are associated with invasive signaling pathways.[23] In gallbladder cancer, elevated CCL2 expression enhanced invasion, and the increased CCR2 and CCL2 serum levels were linked to lung metastasis in mouse models.[24] Overall, these findings underscore the fundamental role of CCL2 function in cancer progression and metastasis across various tumor types.

Additionally, stratified analysis by tumor grade showed a clear trend of increasing serum levels of IL-8, CXCL12, and CCL2 with higher tumor grades (I to III). This trend reinforces the contribution of these chemokines in tumor aggressiveness and may indicate their role in the malignant transformation of ependymomas. Specifically, IL-8 levels were markedly elevated in grade III tumors, underscoring its involvement in angiogenesis and immune evasion. Similarly, CXCL12 and CCL2 were substantially higher in higher-grade tumors, suggesting their importance in cell proliferation and the recruitment of tumor-associated immune cells. These findings support the prognostic relevance of chemokine profiling in clinical management and risk stratification of spinal ependymoma.

Comparative analyses of chemokine expression profiles between spinal and intraventricular ependymomas reveal notable differences that underscore the unique microenvironmental characteristics of spinal tumors. Spinal ependymomas exhibit elevated expression of chemokines such as CCL2, which is associated with the recruitment of monocytes and tumor-associated macrophages, contributing to an immunosuppressive tumor microenvironment.[25] In contrast, intracranial ependymomas, particularly those located in the posterior fossa, demonstrate a pro-inflammatory microenvironment characterized by the expression of MHC class II-related molecules and other pro-inflammatory cues.[26] These differences in chemokine expression and immune cell infiltration patterns highlight the distinct biological behaviors of ependymomas based on their anatomical location, which may have implications for prognosis and therapeutic strategies.

Genetic polymorphisms in the IL-8 gene promoter region, notably rs4073 (IL-8 -251T>A), have been associated with variations in IL-8 expression levels and susceptibility to various inflammatory and neoplastic diseases in Asian populations. For instance, a meta-analysis encompassing 33 case-control studies revealed that the IL-8-251A allele is significantly associated with an increased risk of gastric cancer in Asian populations, including Chinese and Korean individuals.[27] Similarly, studies have demonstrated associations between IL-8 polymorphisms and elevated risks of other cancers in Asian populations.[28] These findings suggest that genetic variations in IL-8 may contribute to the observed differences in chemokine levels and disease susceptibility among Asian populations. Further genetic analyses are warranted to elucidate the role of these polymorphisms in the pathogenesis and progression of spinal ependymomas.

There are several limitations to the current study. First, while the 50 patients with spinal ependymoma and 25 healthy controls are adequate for a preliminary report, the sample size may be insufficient to generalize the results to a larger population. Additionally, the study design does not allow the researchers to conclude the long-

term prognostic value of IL-8, CXCL12, and CCL2, as no longitudinal data were collected. In addition, this study found associations between chemokine and clinical features but could not uncover the molecular mechanisms behind the associations of chemokine with the tumors of high aggressiveness, thereby leading to a lack of insight into the role of chemokine in tumor progression. The analyses failed to fully account for potential confounders, including comorbidities, concurrent treatments, or genetic variants that might affect the observed outcomes. The investigation additionally only highlighted serum biomarkers, which may not necessarily reflect the tumor microenvironment, nor was it functional to show that these chemokines drive malignancy. Future studies should focus on longitudinal follow-up, mechanistic exploration, and tissue-based analyses to better define the role of these specific chemokines in spinal ependymoma.

In conclusion, this study highlights the significant elevation of serum IL-8, CXCL12, and CCL2 levels in patients with spinal ependymoma compared to healthy controls. In particular, IL-8 showed significant correlations with tumor size, grade, and KPS score, highlighting its potential role as a prognostic biomarker for spinal ependymoma. Contrastingly, CXCL12 and CCL2 exhibited a highly significant association with tumor size and grade, thereby reinforcing the hypothesis of their involvement in tumor progression. The observations described above reveal information about the chemokine-driven inflammatory milieu of spinal ependymoma and the implications for tumor behavior. These chemokines may serve as novel biomarkers for the diagnosis, prognosis, and therapeutic targeting of spinal ependymoma.

DISCLOSURE

Ethics: The Research Ethics Committees of Islamic Azad University-Arsanjan Branch approved the study (number: IR.IAU.A.REC.1403.025). All participants provided written informed consent.

Financial support: None

Conflict of interest: None

REFERENCES

1. Lampros M, Vlachos N, Alexiou GA. Ependymomas in children and adults. *Adv Exp Med Biol* 2023; 1405;99-116. DOI: 10.1007/978-3-031-23705-8_4
2. Zhang XW, Wu XY, Sheng XF, *et al*. Ependymoma diagnosis and treatment progress. *Int J Clin Exp Med* 2016;9(8):15050-7.
3. Rudà R, Reifenberger G, Frappaz D, *et al*. EANO guidelines for the diagnosis and treatment of ependymal tumors. *Neurooncology* 2018;20(4):445-56. DOI: 10.1093/neuonc/nox166
4. Mir MA, Rashid M, Jan N. Cytokines and chemokines in tumor growth and progression. Cytokine and Chemokine Networks in Cancer: Springer; 2023. 33-77. DOI:10.1007/978-981-99-4657-0_2
5. Matsushima K, Yang D, Oppenheim JJ. Interleukin-8: An evolving chemokine. Cytokine 2022;153:155828. DOI: 10.1016/j.cyto.2022.155828
6. Fousek K, Horn LA, Palena C. Interleukin-8: A chemokine at the intersection of cancer plasticity, angiogenesis, and immune suppression. Pharmacol Ther 2021;219:107692. DOI: 10.1016/j.pharmthera.2020.107692.
7. Najdaghi S, Razi S, Rezaei N. An overview of the role of interleukin-8 in colorectal cancer. Cytokine 2020;135:155205. DOI: 10.1016/j.cyto.2020.155205
8. Liu H, Zhao Q, Tan L, *et al*. Neutralizing IL-8 potentiates immune checkpoint blockade efficacy for glioma. Cancer cell 2023;41(4):693-710. e8. doi: 10.1016/j.ccell.2023.03.004.
9. Yoshimura T, Li C, Wang Y, Matsukawa A. The chemokine monocyte chemoattractant protein-1/CCL2 is a promoter of breast cancer metastasis. Cell Mol Immunol 2023;20(7):714-38. DOI: 10.1038/s41423-023-01013-0
10. Shao Y, Han S, Hou Z, Yang C, Zhao Y. Tumor-associated macrophages within the immunological milieu: An emerging focal point for therapeutic intervention. Heliyon 2024;10(17). DOI: 10.1016/j.heliyon.2024.e36839
11. Shi Y, Riese DJ, Shen J. The role of the CXCL12/CXCR4/CXCR7 chemokine axis in cancer. Front Pharmacol 2020;11:574667. DOI: 10.3389/fphar.2020.574667
12. Cambier S, Gouwy M, Proost P. The chemokines CXCL8 and CXCL12: molecular and functional properties, role in disease and efforts towards pharmacological intervention. Cell Mol Immunol 2023;20(3):217-51. DOI: 10.1038/s41423-023-00974-6
13. Rizzo M, Varnier L, Pezzicoli G, Pirovano M, Cosmai L, Porta C. IL-8 and its role as a potential biomarker of resistance to anti-angiogenic agents and immune checkpoint inhibitors in metastatic renal cell carcinoma. Front Oncol 2022;12:990568. DOI: 10.3389/fonc.2022.990568
14. Zippoli M, Ruocco A, Novelli R, *et al*. The role of extracellular vesicles and interleukin-8 in regulating and mediating neutrophil-dependent cancer drug resistance. Front Oncol 2022;12:947183. DOI: 10.3389/fonc.2022.947183
15. Teijeira A, Garasa S, Ochoa MC, *et al*. IL8, neutrophils, and NETs in a collusion against cancer immunity and immunotherapy. Clin Cancer Res 2021;27(9):2383-93. DOI: 10.1158/1078-0432.ccr-20-1319

16. Garg P, Jallepalli VR, Verma S. Unravelling the CXCL12/CXCR4 Axis in breast cancer: insights into metastasis, microenvironment interactions, and therapeutic opportunities. Human Gene 2024:201272. DOI: 10.1016/j.humgen.2024.201272
17. Roberto M, Arrivi G, Di Civita MA, *et al*. The role of CXCL12 axis in pancreatic cancer: New biomarkers and potential targets. Front Oncol 2023;13:1154581. DOI: 10.3389/fonc.2023.1154581
18. Zhang JJ, Liu W, Xing GZ, Xiang L, Zheng WM, Ma ZL. Role of CC-chemokine ligand 2 in gynecological cancer. Cancer Cell Int 2022;22(1):361. DOI: 10.1186/s12935-022-02763-z
19. Kitamura T, Qian BZ, Soong D, *et al*. CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. J Exp Med 2015;212(7):1043-59. DOI: 10.1084/jem.20141836
20. Jin J, Lin J, Xu A, *et al*. CCL2: an important mediator between tumor cells and host cells in tumor microenvironment. Front Oncol 2021;11:722916. DOI: 10.3389/fonc.2021.722916
21. Xu M, Wang Y, Xia R, Wei Y, Wei X. Role of the CCL2-CCR2 signalling axis in cancer: mechanisms and therapeutic targeting. Cell Prolif 2021;54(10):e13115. DOI: 10.1111/cpr.13115
22. Cai Q, Yang J, Shen H, Xu W. Cancer-associated adipocytes in the ovarian cancer microenvironment. Am J Cancer Res 2024;14(7):3259. DOI: 10.62347/XZRI9189
23. Mohamed HT, El-Ghonaimy EA, El-Shinawi M, *et al*. IL-8 and MCP-1/CCL2 regulate proteolytic activity in triple negative inflammatory breast cancer a mechanism that might be modulated by Src and Erk1/2. Toxicol Appl Pharmacol 2020;401:115092. DOI: 10.1016/j.taap.2020.115092
24. Zhuang H, Cao G, Kou C, Liu T. CCL2/CCR2 axis induces hepatocellular carcinoma invasion and epithelial-mesenchymal transition in vitro through activation of the Hedgehog pathway. Oncol Rep 2018;39(1):21-30. DOI: 10.3892/or.2017.6069
25. Zhang Q, Cheng S, Wang Y, *et al*. Interrogation of the microenvironmental landscape in spinal ependymomas reveals dual functions of tumor-associated macrophages. Nat Commun 2021;12(1):6867. DOI: 10.1038/s41467-021-27018-9
26. Aubin RG, Troisi EC, Montelongo J, *et al*. Pro-inflammatory cytokines mediate the epithelial-to-mesenchymal-like transition of pediatric posterior fossa ependymoma. Nat Commun 2022;13(1):3936. DOI: 10.1038/s41467-022-31683-9
27. Moghimi M, Dastgheib SA, Heiranizadeh N, Zare M, Sheikhpour E, Neamatzadeh H. Association of IL-8-251T> A (rs4073) polymorphism with susceptibility to gastric cancer: A systematic review and meta-analysis based on 33 case-control studies. Arq Gastroenterol 2020;57(01):91-9. DOI: 10.1590/S0004-2803.202000000-16
28. Wang XB, Li YS, Li J, Han Y, Liu ZD. Interleukin-8-251A/T gene polymorphism and lung cancer susceptibility: a meta-analysis. J Cell Mol Med 2015;19(6):1218-22. DOI: 10.1111/jcmm.12466