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Research Article

# CXCL family of chemokine: Clinical perspective in colorectal cancer

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**Abstract**
**Background:** Chemokine are small proteins which play a critical role in colorectal pathophysiology by stimulating inflammation, influencing angiogenesis and movement of immune cells. Recent research has emphasized the possible role of CXC chemokine family in influencing cancer development. In this study, focus was to investigate the CXC chemokines as potential biomarkers in colorectal cancer by comparing their levels in primary phases.
**Methods:** This comparative cross-sectional study was conducted while using serum samples of colorectal cancer patients (stage II & III) and healthy controls. The circulatory levels of CXCL chemokines were quantified by using enzyme-linked immunosorbent assay (ELISA) and differences between chemokine levels of colorectal cancer patients and healthy controls were analyzed using an independent t-test via the latest version of SPSS.
**Results:** The study findings showed that concentrations of CXCL1, CXCL9, and CXCL11 chemokine were raised considerably with colorectal cancer progression, particularly in stage III, indicating their involvement in cancer progression. CXCL10 and CXCL11 concentrations were found to be significantly altered between the two stages of colorectal cancer ($p<0.05$).
**Conclusion:** CXCL levels de-regulate in colorectal cancer and may serve as biomarkers and therapeutic targets.
**Key Words:** Colorectal cancer, Chemokine, Expression, Serum levels

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## INTRODUCTION

Colorectal cancer (CRC) ranks among the major health problems around the world. It is the 3rd most prevalent cancer and the 2nd leading cause of mortality globally. By the year 2030, this disease is estimated to cause around 2.2 million new cases and almost 1.1 million fatalities annually [1, 2] and by the year 2040, the global incidence of this disease is projected to become more than double [3]. The pathophysiology of CRC is complex, involving genetic, immunological and lifestyle-related factors such as diet and smoking [4].

Chemokines have emerged as critical mediators in tumor development, immunological evasion, and metastasis of CRC. Particularly the CXC chemokine family, which includes CXCL1, CXCL8, CXCL9, CXCL10, and CXCL11, has captured great scientific attention due to their involvement in CRC biology and potential as biomarkers for disease progression and therapeutic targets. In addition, therapy resistance is a major contributor to poor rates of survival in CRC, emphasizing the need for new reliable biomarkers to improve the possibilities for treatment [5].

Chemokines are tiny, secreted proteins from the cytokine superfamily. Its fundamental role is within the tumor microenvironment (TME) providing facilitation for infiltration and movements of specific immune system cells. These molecules are typically short peptides that range in molecular weight from 8 to 14 kDa and play an important role in influencing immunological responses in numerous clinical conditions, especially cancer [6].

Till now, over 50 chemokines have been identified and classified into four major subgroups known as the CC chemokines, CXC chemokines, CX3C chemokines, and C chemokines depending on the location of cysteine residues. The CXC chemokine family has 17 identified members till now that primarily bind with CXC motif chemokine receptors (CXCRs). These chemokines exhibit distinct effects on angiogenesis, a critical step in the development of cancer and metastasis. CXCL1 and CXCL8, which contain a second ELR motif, stimulate angiogenesis by interacting with the CXCR2 receptor. Conversely, all the other three chemokines (CXCL9, CXCL10, CXCL11) produce angiostatic effects by directly interacting with the CXCR3 receptor [7]. Despite their renowned functions in different cancers, there is insufficient knowledge regarding the clinical relevance of these tiny substances as potential biomarkers for CRC. Studying the variations of chemokine levels in CRC could offer a glimpse at their potential as diagnostic/ prognostic biomarkers and as potential therapeutic targets.

## METHODS

### Clinical Sampling

A total of 3-5 ml of blood from 80 participants, which includes 40 healthy controls, 22 stage II, and 18 stage III CRC patients were collected. Blood samples were collected under aseptic settings with sterile syringes and transferred to BD serum separator vials. After a 30-minute coagulation period at room temperature, specimens were centrifuged at 1000 x g for 15 minutes to separate the serum. The serum was kept in nuclease-free vials at ≤ -20°C for further processing.

### ELISA

Serum chemokine levels were measured utilizing a multi-analyte ELISArray (Qiagen), based on sandwich-based ELISA technique. A 96-well microplate, pre-coated with capture antibodies against chemokine enabled us for simultaneous analysis of samples. The serum specimens were divided into three categories: stage II, stage III CRC and healthy controls. A total of 50µl aliquots from every specimen were dispensed into distinct tubes for testing. Every pooled serum specimen in duplicate was thoroughly diluted in the assay buffer before loading into corresponding wells inside the ELISArray plate. Following the manufacturer's instructions, absorbance readings were measured at 450 nm wavelength utilizing an ELISA plate reader. SPSS was used to analyze the data, independent t-test was employed to find the difference in chemokine level between the two groups. p-value of <0.05 was considered statistically significant.

## RESULTS

### CXCL chemokine in colorectal cancer patients

The study highlighted circulatory levels of CXCL chemokine in serum samples of CRC patients in comparison to healthy controls. Mean serum concentration of CXCL1 (GRO-α) was 22pg/ml in stage II, 34pg/ml in stage III, and 25pg/ml in healthy controls. The findings demonstrated an increase in CXCL1 levels in advanced stages of CRC (stage III), even though there was some reduction in early CRC (stage II). CXCL9, CXCL10, and CXCL11 are chemokine ligands that trigger T-cell chemotaxis through a shared receptor, CXCR3. CXCL9 showed a gradual increase in circulation during CRC development. Average concentration of CXCL9 was 43pg/ml in healthy controls, 49pg/ml in stage II, and 68pg/ml in stage III CRC patients. The average concentration of CXCL10 chemokine (also known as IP-10), was almost similar in healthy controls, stage II, and stage III patients. However, a statistically significant difference of CXCL10 concentration was seen in stages II and III of CRC ($p < 0.05$). Precisely, CXCL10 concentrations in healthy controls and CRC patients (stage II & III) were 98pg/ml, 91pg/ml and 108pg/ml, respectively. Patients with CRC showed an overall rise in CXCL11 (I-TAC) chemokine. Moreover, this increase was significant with each successive stage of CRC ($p<0.05$). The average CXCL11 concentration was 14pg/ml in healthy controls, 19pg/ml in stage II, and 27pg/ml in stage III CRC patients. Overall changes in the selected chemokine in CRC patients are shown in Figure 1.

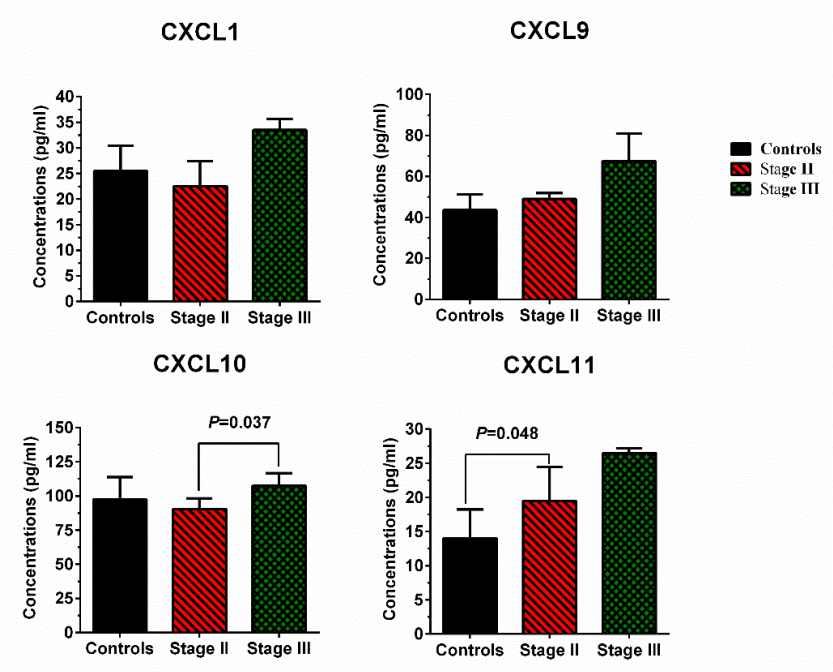


**Figure 1:** Circulatory levels of CXCL chemokine in colorectal cancer patients and healthy controls.

## DISCUSSION

The differential expression of the chemokine pathway in diseased conditions is well recognized. Depending on their type, kind, concentration, and tissue involvement, chemokine may play support or hinder the cancer progression. In cancer, this complicated network governs immune cell infiltration, tumor development, survival, and migration. Numerous cells, including immunological, stromal, and tumor cells, express and respond to chemokine variations. Despite substantial research, specific chemokine profiles for certain cancer types remain unknown, particularly in terms of circulatory levels. Given the chemokine network's functional involvement in cancer, investigations are needed regarding their circulating levels assessments and subsequent effects. To add knowledge, present study was designed to uncover the levels of CXCL chemokine while using clinical isolates.

Over 90% of patients enrolled in this study had advanced CRC (stage II and III), likely due to asymptomatic disease, late diagnosis and lack of early screening. To ensure statistically significant results, we focused on stage II and III samples only. Chemokine levels were measured using an ELISArray plate coated with antibodies for each chemokine, and results were compared with healthy controls. The selected ELISA kit enabled us to determine the levels of multiple chemokines at the same time from the selected samples. Distinct changes in chemokine concentrations were seen in CRC patients as compared to healthy controls. However, these variations were statistically significant ($P \leq 0.05$) for two chemokine (CXCL10, CXCL11). As we know, CXCL1 plays a critical role in angiogenesis along with the movement of tumor cells that augment tumor growth. Wang, D et al., reported enhanced levels of CXCL1 in CRC tumor cells and further explained that CRC cells also release VEGF and FGF, other chemokine resulting in neovascularization in *in vivo* [8]. In this study, a fractional up-regulation of CXCL1 was observed in stage III CRC patients, which in turn might promote progression, especially growth at secondary sites during metastasis [9]. A study revealed function of CXCL1 in recruiting suppressor cells myeloid-derived cells (MDSCs), which showed that CXCL1 promotes CRC metastasis by increasing MDSC infiltration [10]. Another study reported that the expression of CXCL1 was significantly higher in CRC patients as compared to the expression of healthy controls [11]. A study by YU et al. suggested that the upregulation of CXCL1 and CXCL8 in CRC tissues may have an important role in the carcinogenesis of CRC [12] which aligns with the findings of our study.

CXCL9, 10, and 11 play key roles in overall CRC development, progression, and metastasis. Our data showed elevated levels of CXCL 9 and 11 in CRC patients as compared to healthy individuals. In contrast, minimal alterations in the expression level of CXCL10 were noticed when comparing CRC patients with healthy controls. These findings are strengthened by the previous studies that also reported high expression of CXCL9 and 11 and are linked with high infiltration of macrophages, CD4+, and CD8+ memory T-cells that ultimately help in prognosis. CXCL10 levels are reduced in CRC patients mostly in the CRC cases with recurrence when compared to the ones without recurrence [16-17]. Moreover, a study by Li *et al.,* reported that expression of CXCL10 was significantly elevated in patients suffering from advanced CRC, which is consistent with our findings [18]. In conclusion, chemokine levels were distinctly expressed in CRC patients as compared to normal controls, with higher circulatory levels visible in picograms. These high levels could be used as tumor or prognostic markers that would help in cancer diagnosis and treatment. Given their crucial roles in carcinogenesis, chemokines also present promising targets for therapeutic interventions.

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**Ethics Approval:** Study was approved by the Ethical Review Committee, University of Health Sciences, Lahore, Pakistan.

**Author Contributions:** Syeda Faiza collected the samples and performed the ELISA experiments. Asim Pervaiz supervised the manuscript draft.

**Competing Interests:** The authors declare that they have no conflict of interest.

**Data Availability Statement:** Datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.