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Expression and Clinical Significance of the Chemokine Receptor CXCR2 in Ovarian Cancer

Maysaa G. Jumaah

College of medicine / University of Missan / Missan / Iraq.

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Abstract: The present study aimed to shed light on the role the of the Chemokine Receptor CXCR2 in the pathogenesis and progression of ovarian cancer. A total of 23 Paraffin-embedded tissue blocks from patients with different stages of newly diagnosed ovarian cancer were provided by certain Iraqi hospitals as well as 7 samples of patients with benign ovarian tumors tissues as a control group were used in this study. In the present study, the CXCR2expression was assessed by means of an envision immunohistochemistery technique using the Novolink^{TM} Polymer Detection Systems for both benign and malignant ovarian tumors. The results showed that 6(85.7%) of benign ovarian tumors and 22(95.7%) of ovarian cancer samples were positive for CXCR-2 which showed significant differences (P value0.048<0.5). CXCR2 was not expressed in 1(14.3%) of benign tumors and 1(4.3%) of ovarian cancer sections. Weak (+) CXCR-2 expression was observed in 2(28.5%) of benign tumors and 5(21.7%) of ovarian cancer sections which showed no significant differences. No significant differences were observed in the median CXCR-2 expression (++) between samples of benign tumors 3(42.8)% and samples of ovarian cancer 5(21.73%). The highest vascular signal intensity of CXCR-2 expression (+++) was observed in 1(14.3%) of benign tumors and 12(52.1%) of ovarian cancer sections, which showed high significant differences (P value **<0.01). In correlation with stages the results showed that18(94.44%) of samples with stage I and 4(100%) of samples with stage III were positive for CXCR2, which showed no significant differences (NS) with differences I signal intensity. In conclusion this study investigated that the percentages of sections with positive expression were higher in ovarian cancer tissue sections than the sections of benign ovarian tumors, and the signal intensity of staining was stronger in late stages of ovarian cancer tissue indicate role of CXCR2 expression in ovarian tumor progression, and maybe reveals the diagnostic value of this receptor for early diagnosis of ovarian cancer, and also provide the evidence for the ability of tumor cells to metastasize and then tumor angiogenesis and invasiveness.

Keywords: Ovarian cancer, Immunohistochemistery, IL-8, Chemokines receptors, CXCR2.

Corresponding author: (Email: maisaagazi@yahoo.com).

Introduction:

Ovarian cancer is the most lethal gynecologic malignancy, unfortunately it is fatal in the majority of cases. The lifetime risk of ovarian cancer is approximately 2% and it is the fourth most common cause of cancer-related death in women within the United Kingdom, although its incidence worldwide is slightly less (1,2). In 2005, almost 7000 cases of ovarian cancer were diagnosed, and until recently it was the most common gynaecological malignancy. Current data illustrates that it is now the second commonest after uterine cancer. The incidence of ovarian cancer has remained constant, but there has been an exponential rise in the rates of endometrial cancer. Ovarian cancer is predominately a disease of older, postmenopausal women. The incidence increases rapidly after the age of 50, with over 85% of ovarian cancers occurring above that age (3).

Chemokine receptor type 2 (CXCR2) is a G protein-coupled receptors. Various cancer cell types have the ability to secreted and expressed the chemokines for CXCR1/2 including CXCL1, 5, 7, and 8, which stimulate cancer cell migration and proliferation in an autocrine fashion (4). Overexpression of CXCL1-3 and CXCL8 is associated with vascular invasive cancer phenotype, while inhibition of CXCR2 signaling reduces tumor invasion(5). CXCR1 and CXCR2, are only overexpressed in very few cancer cell lines, in contrast to the CXCL8 which overexpressed in various cancer cell lines. The previous studies assumed that the knockdown negatively impacted cell survival and proliferation, is represent the functional importance of the CXCL8-CXCR1/2(6).

The capacity of tumor to induce angiogenesis play the key role in tumors progression beyond 2-3 mm^3 (7). The induction of tumor angiogenesis and endothelial cells expressing of CXCR1/2 stimulated by CXCR1/2 ligands. Several in vivo cancer models were used to demonstrate the involvement of CXCR2 in tumor progression and angiogenesis, which showed that the depletion of chemokines and/or the receptor significantly reduced tumor growth associated with decreased microvessel density (8). In this study, we assessed expression of CXCR2 in human benign and malignant ovarian tumors and explored the relationship of CXCR2 in tumor progression.

Materials and Methods:

Subjects and samples collection:

The tissue samples used in this study included 23 Paraffin-embedded tissue blocks from patients with different stages of newly diagnosed. Invasive ovarian cancer were provided by certain Iraqi hospitals (including Al-Kadhemia , AL -Yarmouk Teaching Hospital, Baghdad Hospital, the Teaching Laboratories of Medical City, Nuclear Medical Hospital in Baghdad and Alsader Hospital in Missan) after patients underwent to total abdominal hysterectomy and bilateral salpangiooopherectomy (TAH-BSO), subtotal abdominal hysterectomy, vaginal hysterectomy, and endometrial biopsy, 7 samples of patients with benign ovarian tumors tissues were used as a control. The required information about the patients and the histopathologic properties of the tumors were recorded from the patients' files.

Preparation of tissuesections and Immunohistochemical Detection of CXCR2:

The tissues sectioning and technique validation were performed at the Liver Unit / King's College Hospital / London, while the immunohistochemical detection of CXCR2 was performed at the Oralpathology laboratory in Guy's and St Thomas's Hospital /London/UK. Paraffin embedded sections of benign and malignant ovarian tumors were cut into 4 µm thicknesses using a microtome. The sections were applied on Fisher-brand positively chargedslides. The tissue expression of CXCR2 was assessed by means of an envision technique using the Novolink™ Polymer Detection Systems which provide by using commercial kit from Novocastra, (Newcastle, UK). The

envision technique were used for the visualization of mouse IgG, mouse IgM and rabbit IgG primary antibodies. The positive cells were visualized by using a polymeric conjugate system to recognize mouse and rabbit immunoglobulins. Polymer-based immune his to chemical methods utilize a technology based on a polymer backbone to which multiple antibodies and enzyme molecules are conjugated. In this technique (polymer system), 70 enzyme molecules and about 10 antibodies were conjugated to dextran backbone. This allows the entire immune his to chemical staining procedure, from primary antibody to enzyme, to be accomplished in a single step. The respective presence and absence of CXCR2 in tumor vasculature and tumor cells were confirmed by comparing the receptor expression in a selection of seven sections of benign tumors and twenty three sections of ovarian cancer with positive control sections (tonsils tissues forCXCR2). For each tissue sample, the maximum CXCR2 staining intensity was recorded on an ordinal scale (0, +, ++, +++). Separate intensities were recorded for the benign and malignant tumors, different types of ovarian cancer, and stage I and stage III of ovarian cancer.

Statistical Analysis:

The Statistical Analysis System-SAS was used to effect of difference factors in study parameters or percentage. The chi-square test at the comparative between percentages in this study.

Results and Discussion:

The results of CXCR2expression showed in (Table 1). The results showed that 22(95.7%) of ovarian cancer and 6(85.7%) of benign ovarian samples were positive for CXCR-2 which showed significant differences (P value0.048<0.5). CXCR2 was not expressed (-) in 1(14.3%) of benign tumors and 1(4.3%) of ovarian cancer sections. Weak (+) CXCR-2 expression was observed in 2(28.5%) of benign tumors and 5(21.7%) of ovarian cancer sections which showed no significant differences (P value 0.0631), (Figure 1). No significant differences (P value 0.0722 NS) were observed in the median CXCR-2 expression (++) between samples of benign tumors 3(42.8%) and samples of ovarian cancer 5(21.73%), (Figure 2). The highest vascular signal intensity of CXCR-2 expression (+++) was observed in 1(14.3%) of benign tumors and 12(52.1%) of ovarian cancer sections, which showed high significant differences (P value 0.0028 **<0.01), (Figure 2). In correlation with stages the results showed that 18(94.44%) of samples with stage I and 4(100%) of samples with stage III were positive for CXCR2, which showed no significant differences (P value 0.894NS). The results showed that the weak signal intensity (+) of CXCR2 expression was significantly higher in stage I than stage III (P value 0.0015**<0.01). There were no significant differences (P value 0.892 NS) in the median (++)CXCR2 expression between the two stages. High significant differences in the highest vascular signal intensity of CXCR2 expression (+++) in stage III 4(80%) compared with 10(55.5%) with stage I (P value 0.0027**<0.01).

Table (1): Percentage of CXCR-2 signal intensity in ovarian cancer patients with stage I andIII.

Group ofpatients	No. of patients	Negative%	Positive%	Pvalue	
Benigntumors	7	(1)14.3%	(6)85.7%		
Ovariancancer	23	(1)4.3%	(22)95.7%	0.048 *	
Signalintensity	-%	+%	++%	+++%	
Benigntumors	(1)14.3%	(2)28.5%	(3)42.8%	(1)14.3%	
Ovariancancer	(1)4.3%	(5)21.7%	(5)21.7%	(12)52.1%	
Pvalue	0.619NS	0.0631NS	0.0722NS	0.0028 **	
Ovarian cancerStages		Negative%	Pvalue	Positive%	Pvalue
I(19)		(1)5.55%		(18)94.44%	
III(4)		0.00%	0.891NS	(4)100%	0.894NS
Signalintensity		Stages	+%	++%	+++%
		I(18)	(5)27.7%	(3)16.6%	(10)55.5%
		III(5)	0.00	(1)20.00%	(4)80.00%
Pvalue			0.0015**	0.892NS	0.0003 **

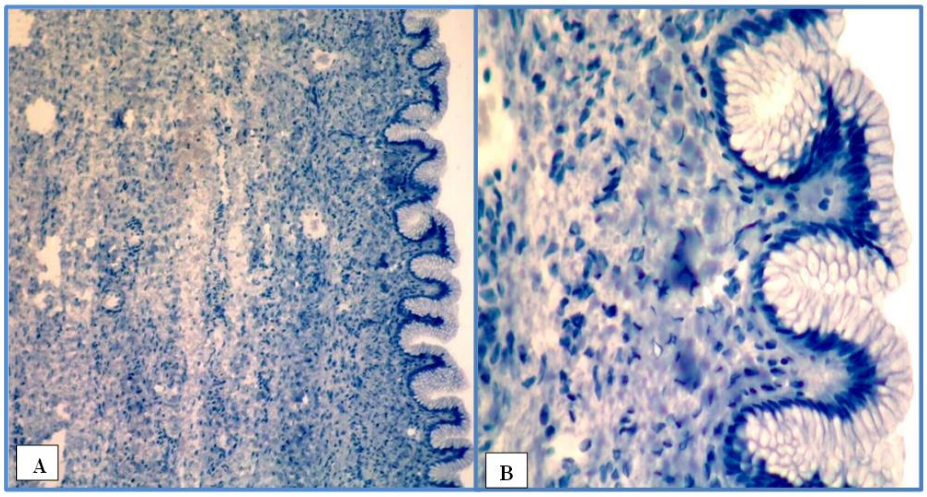

Figure (1): Benign ovarian tumor, showing no expression(-) for CXCR2, (A/20 X,B/40X).

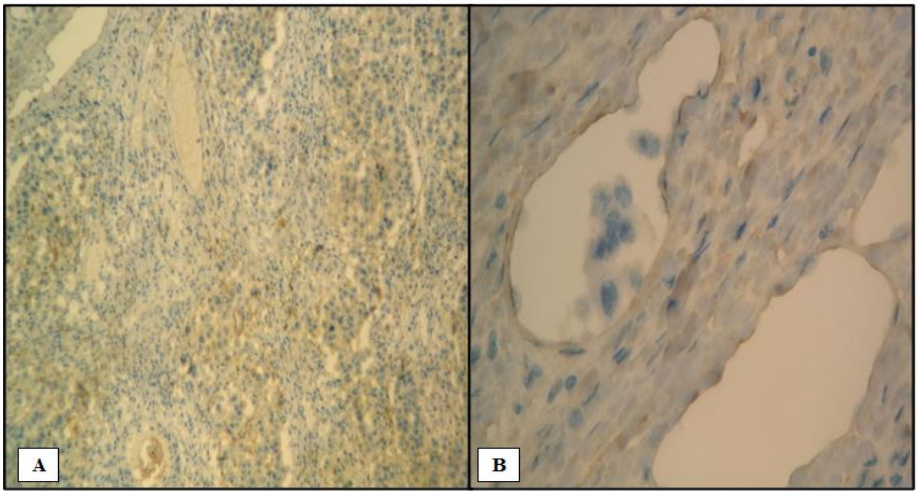

Figure (2): Germ cell tumor/dysgerminoma tumor, Well differentiated, Stage I, showing low expression(+) of CXCR2, (A/20 X,B/40X).

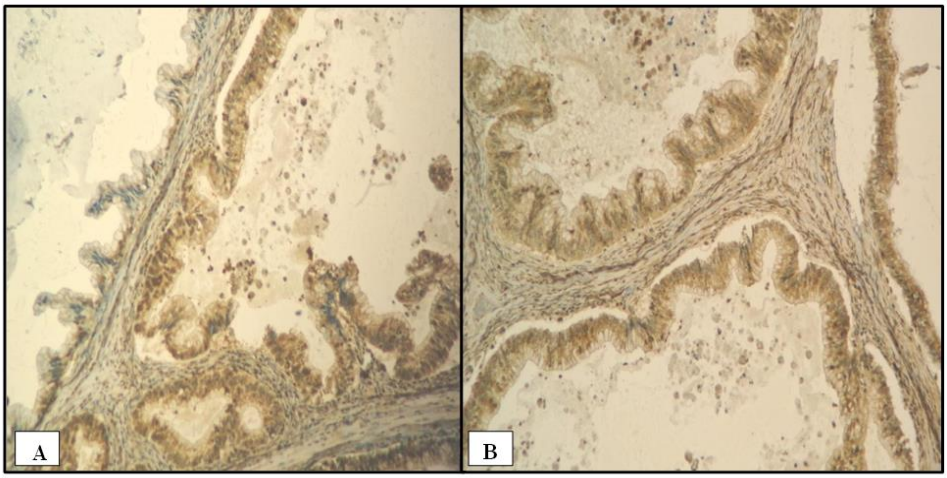

Figure (3): Mucinous cyst adenocarcinoma Well differentiated, Stage I, showing moderate expression(++) of CXCR2, (A/20 X,B/40X).

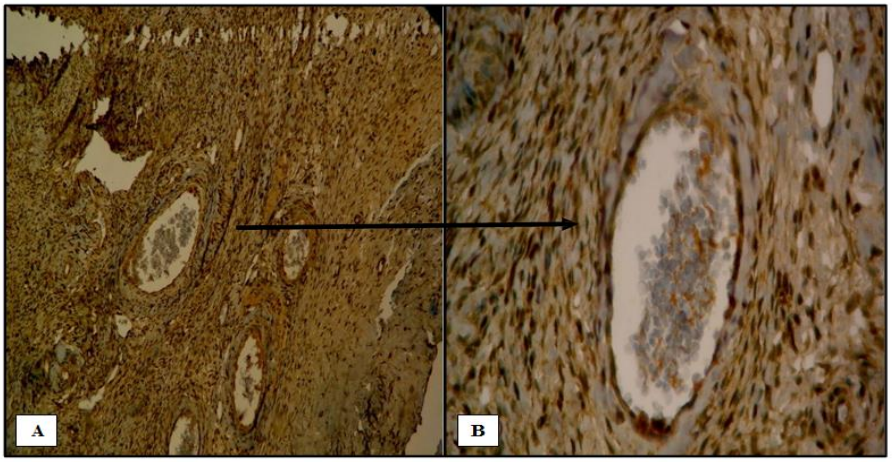

Figure (4): Endometrial adenocarcinoma, Poorly differentiated, Stage III, showing High expression (+++) of CXCR2, (A/20 X,B/40X).

It is well known that upregulation of IL-8 promotes cell proliferation and survival (9), but how the chemokines and their receptor regulate cell cycle progression and apoptosis have been little investigated. CXCR2 plays a critical role in ovarian cancer progression by regulating the cell cycle, apoptosis, and angiogenesis via multiple signaling pathways, including PI3K/AKT, NF-κB, MAPK, and STAT3. The results of the present study showed 95.7% of ovarian cancer samples were positive for CXCR-2 which showed significant differences (P value0.048<0.5). Huang *et al.,*(10) found no difference in the expression of IL-8 receptors between benign and malignant neuroendocrine (NE) cells. While Cheng *et al*.,(11) showed that CXCR2 expression was higher in gastric cancer tissues compared with adjacent noncanceroustissues, Yang *et al.*,(12) reported that no expression of CXCR2 was detected in normal epithelial tissues. In correlation with malignancy, CXCR2 was not expressed (-) in 14.3% of benign tumors and 4.3% of ovarian cancer sections. Weak (+) CXCR-2 expression was observed in

14.3% of benign tumors and 21.7% of ovarian cancer sections 2 which showed no significant differences (P value 0.0631).No significant differences (P value 0.0722 NS) were observed in the median CXCR-2 expression between samples of benign tumors 28.5% and samples of ovarian cancer 21.73%. The highest vascular signal intensity of CXCR-2 expression (+++) was observed in 14.3% of benign tumors and 52.1% of ovarian cancer sections, which showed high significant differences (P value 0.0028 **<0.01). Yang et al.,(13) showed that (30.8%) of human serous ovarian carcinoma specimens had high CXCR2 expression, (38.5%) had moderate expression, and the remaining (30.8%) had nearly undetectable expression, whereas little CXCR2 expression was detected in normaltissues. In correlation with stages the results showed that (94.44%) of samples with stage I and (100%) of samples with stage III were positive for CXCR2. Similar results were recorded by Yang *et al*. (12) who found that CXCR2 was highly expressed in late stage than the early stage of ovarian cancer. The results showed that the weak signal intensity (+) of CXCR2 expression was significantly higher in stage I than stage III (P value 0.0015**<0.01). CXCR2 plays a critical role in ovarian cancer progression by regulating the cell cycle, apoptosis, and angiogenesis via multiple signaling pathways, including PI3K/AKT, NF-κB, MAPK, and STAT3. Some studies showed that CXCR2 is overexpressed in both ovarian cancer cell lines and ovarian cancer from patients but not in normal cells and tissues, suggesting that CXCR2 may be a potential target for ovarian cancer treatment. Antagonists against CXCR2 have been developed to inhibit various types of tumor growth. Thus, to develop some antagonists of CXCR2 that can effectively inhibit ovarian cancer cell growth may be a hopeful strategy toward ovarian cancer treatment. Because overexpression of CXCR2 is also associated with a poor survival among ovarian cancer patients, this receptor may be a novel prognostic marker for ovarian cancer and a potential target for therapeutic intervention (12).

Conclusion:

This study investigated that the percentages of sections with positive expression were higher in ovarian cancer tissue sections than the sections of benign ovarian tumors, and the signal intensity of staining was stronger in ovarian cancer tissue sections which indicate role of CXCR2 expression in ovarian tumor progression. The high expression of CXCR2 associated with ovarian cancer tissue sections than the sections of benign ovarian tumors may be reveals the diagnostic value of thisreceptor for early diagnosis of ovarian cancer. On the other hand CXCR2 over expression in late stages is an evidence for the ability of tumor cells to metastasize and then tumor angiogenesis and invasiveness.

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