CORRELATION BETWEEN CHEMOKINE RECEPTOR (CXCR4) EXPRESSION AND CLINICOPATHOLOGICAL FACTORS IN PATIENTS WITH PROSTATE CANCER

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ABSTRACT

Background

Prostate cancer is the second leading cause of cancer-related deaths. Different studies with conatroversial outcomes tried to find a link between CXCR4 level and other clinicopathological characters.

Objectives

To analyze the correlation of CXCR4 with prostate-specific antigen, age of the patient and Gleason score.

Materials and Methods

This is a retrospective cross-sectional study using paraffin-embedded blocks of prostate cancer. For CXCR4 visualization, immunohistochemistry (IHC) based on staining method for demonstration of its level was used to score them according to the extent and the staining intensity of the nucleus as: 0 (no signals for tissue staining), 1 (when tissue extent and intensity for staining is weak), 2 (extent and intensity of tissue staining is moderate), and 3 (when tissue extent and intensity staining is strong).

Results

Age of the patients ranged from 58-88 years, with the P value of 0.874 between the age and IHC staining score. The prostate-specific antigen level ranged from 9-100, the result was non-significant P value of 0.938 between PSA level and IHC staining score. There was also no significant relation between the level of CXCR4 (IHC staining score) and the Gleason score P value of 0.206.

Conclusion

High CXCR4 expression couldn't be related to the clinical parameters such as pretreatment PSA level, the age of the patients, and histopathological findings of prostate cancer using Gleason score.

Keywords: *Prostate cancer, CXCR4, Prostate specific antigen, Immunohistochemistry, Plasma membrane, Transurethral resection of prostate.*

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INTRODUCTION

Prostate cancer (PCa) is the second leading cause of increased cancer incidence and cancer-related deaths among men in the United States [1,2] and one of the top ten leading cancers in Iraq among males according to Iraqi cancer registry in 2009. Several molecules and mechanisms contribute to cancer cell metastasis, for instance, chemoattractant cytokines are chemokines which enhance the metastatic potential of PCa by binding and activating a family of G-protein coupled receptors (GPCRs) that initiate signals to promote cell adhesion, invasion, and movement, and subsequently, tumor survival at the new site of metastasis [3].

An old scheme was established in 1960s by Dr Donald Gleason in USA, for grading prostate adenocarcinoma. He included all the histological growth pattern of prostate adenocarcinoma in relation to staging and also prognosis of the disease. Then in 2004 WHO recommended Gleason score system for prostate cancer [4].

The Gleason system depends on the glandular pattern of the tumor and the cytomorphology of the tumor cells has no role in the grading of the tumor. Both the primary (predominant) and the secondary (second most prevalent) architectural patterns will be graded from 1 to 5. Grade 1 is the most differentiated tumor and 5 shows the least differentiation. The total sum of primary and secondary patterns will be given gleason's score. Tumor with one histologic pattern will be given the same grade. Gleason scores start from 2 (1 + 1 = 2), when the tumors uniformly composed of Gleason pattern 1, and undifferentiated tumors is when the score is 10 (5 + 5 = 10) [5] Table 1.

Table 1. Gleason's microscopic grading system of prostatic carcinoma.

Stage	Description
1	Single, separate, uniform glands in closely packed masses with a definite, usually rounded, edge limiting the area of tumor
2	Single, separate, slightly less uniform glands, loosely packed (separated by small amounts of stroma), with less sharp edge
3a	Single, separate, much more variable glands; may be closely packed but usually irregularly separated; ragged, poorly defined edge
3b	Like 3a, but very small glands or tiny cell clusters
3c	Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumor ('papillary intraductal tumor')
4a	Raggedly outlined, raggedly infiltrating, fused glandular tumor
4b	Like 4a, with large pale cells ('hypernephroid')
5a	Sharply circumscribed, rounded masses of almost solid cribriform tumor, usually with central necrosis ('comedocarcinoma')
5b	Ragged masses of anaplastic carcinoma with only enough gland formation or vacuoles to identify it as adenocarcinoma

Cysteine (C)-X-C Receptor 4 (CXCR4) plays a critical role in prostate cancer metastasis. It is generally regarded as a plasma membrane receptor where transmits signals that support transformation, progression and eventual metastasis. Due to the central role of CXCR4 in tumorigenesis, therapeutic approaches such as antagonist and monoclonal antibodies have focused on receptors that exist on the plasma membrane [6]. Studies have implicated CXCR4 in malignant cancer development by its involvement in cell motility, adhesion, secretion of matrix metalloproteinases (MMPs), angiogenesis and activation of survival signaling pathways [7].

CXCR4 activity is involved in normal homeostasis, such as immune cell migration, embryonic development, growth, angiogenesis, and hematopoiesis. It has been reported that the chemokine CXCR4 was highly expressed in human malignant PCa compared to normal prostate [8, 9]. Hence, several therapeutics for cancer cell metastasis has been designed to antagonize CXCR4-mediated signaling [10].

CXCR4 expression and function and overall tumorigenesis might be enhanced by loss of PTEN function. Loss of PTEN function is typically due to genetic and epigenetic modulations, as well as active site oxidation by reactive oxygen species [11].

If proven to be related, CXCR4 could be a target not only for the development of therapeutic intervention but also for the noninvasive monitoring of PCa progression [12]. In the current study we worked on already diagnosed cases of prostate cancer through paraffin blocks which were performed by prostate biopsy or TURP. Our aim of this study is to analyze the correlation of CXCR4 with prostate specific antigen, age of the patients and Gleason score.

PATIENTS AND METHODS

This is a single center, retrospective cross-sectional study, done in pathology department of College of Medicine, University of Sulaimani, 2017. The enrolled patients' numbers were 40 with prostate carcinoma.

Data collected regarding age of the patients and their pretreatment PSA. Tissue samples (both biopsy and transurethral resection of prostate) obtained by expert urologist of Sulaimani surgical teaching hospital.

The study was done on formalin fixed paraffin embedded blocks. One block was prepared per each patient. For each block 2 slides were prepared as ordinary glass tissue sections, 4µm in size, with H&E stain to determine the Gleason score of the cases.

Gleason system was used for tumor grading, IHC done for all the cases by using charged slides, following the protocol of the Santa Cruz biotechnology company

The basic steps of the IHC-protocol are:

1. Cutting and mounting the section

2. Deparaffinizing the section

3. Antigen retrieval with IHC staining:

CXCR-4 Antibody (4G10) was used, it is a mouse monoclonal IgG1 (kappa light chain) provided at 200 µg/m, raised against the N-terminus amino acids of CXCR-4 of human origin.

Heating slides for 30 minutes at 95°C in citrate buffer (pH 6) to retrieve antigenic activity, then 30 minutes cooling at room temperature.

For the antibody 4G10, slides were incubated for 30 minutes with protein kinase (pH 9) followed by 30 minutes cooling at room temperature.

Slides were rinsed with PBS (Phosphate-buffered saline) and incubated overnight at 4°C with a monoclonal anti-CXCR4-antibody (Santa Cruz Biotechnology Company).

Washing the slides then incubated for 1 hour at room temperature with the secondary antibody, goat-anti-rat (sc-2041; 1:100; Santa Cruz Biotechnology).

Negative controls were only incubated with the secondary antibody.

Washing the slides then incubated for 30 minutes with horseradish peroxidase-labeled streptavidin-biotin complex (1:200; DAKO).

4. Counterstaining with Hematoxylin (Sakura).

5. Dehydrating and stabilizing with mounting medium (DPX/Sakura)

6.Viewing the staining under the microscope.

Immunohistochemistry interpretation: Expressions of CXCR4 were quantified using a visual grading system based on the extent of staining and the intensity of staining (graded on a scale of none (0) when the tissue staining extent and intensity for CXCR4 antibody was negative). For weak response score 1 was used.

Score 2 applied when tissue staining extent and intensity was moderate. Tissue with strong staining and intensity, score 3 was used. Fields at × 10 magnifications were evaluated by the use of an ordinary light microscope (Figure.1).

For data analysis, the data were shown in mean and percentage.Chi-square test was used for comparison of categorical data and t-test for numerical data. A P-value less than 0.05 was regarded as significant.

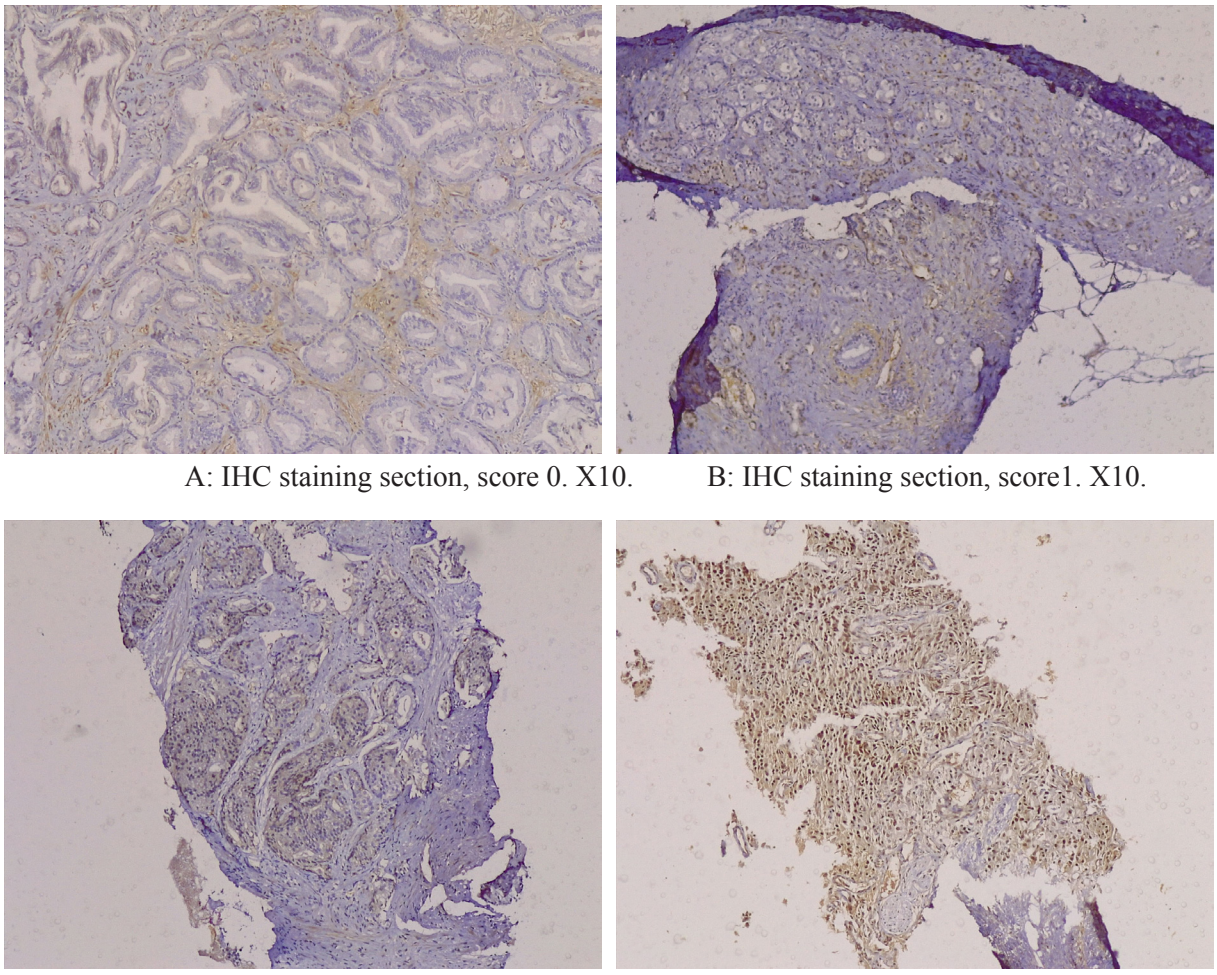
RESULTS

This study was done on 40 paraffin embedded blocks. The ages of the patients ranged from 58-88 years with a mean of 71.85± 7.45. Mean age of the patients according to their relation to the IHC staining intensity (score) was about 73 years with no any staining reaction (0) and score (1), 70 years was the mean for cases score (2), and for score (3) the mean age was 72.3. The P value was 0.8 between the variable of age and IHC staining score. This indicates no relation between IHC staining score and patient's age.

Regarding PSA level, the lowest level was 9 and the highest was 100 with a mean ± SD of 43.63±28.95. Mean of PSA was 35.3 with score (0) IHC staining reaction.

PSA of 48 in score (1), PSA of 43.3 in score (2) and lastly in strong IHC staining score (3) the mean PSA was 43. The association of serum PSA and IHC staining score was not significant (p value 0.938). Figure 2 shows a scatter diagram which indicate individual value of CXCR4 staining level and PSA level for each patient.

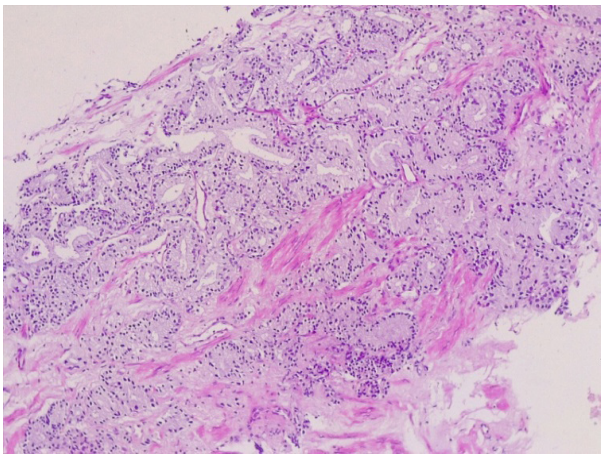
Concerning Gleason score, the lowest score was 4 and only 1 case showed score (2) IHC staining. The highest Gleason score was 9, which includes 5 cases, one of them showed score (0), the others showed score (2) Figure 3, is a scatter diagram that indicates individual value of IHC staining level and Gleason score for patient. The associations of IHC score and Gleason score was also not significant with a p value of 0.206 as shown in Table 2.



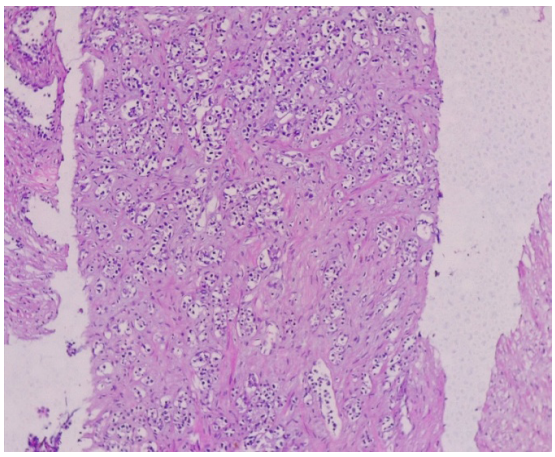
A: IHC staining section, score 0. X10. B: IHC staining section, score1. X10.

C: IHC staining section score2. X10 D: IHC staining section score 3. X10.

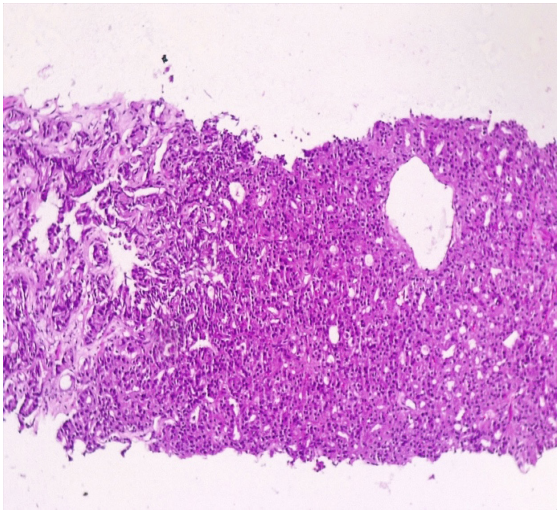
Figure 1. IHC staining score under low power 10x, (A) score 0, (B) score 1 (C) score 2, and (D) score 3.



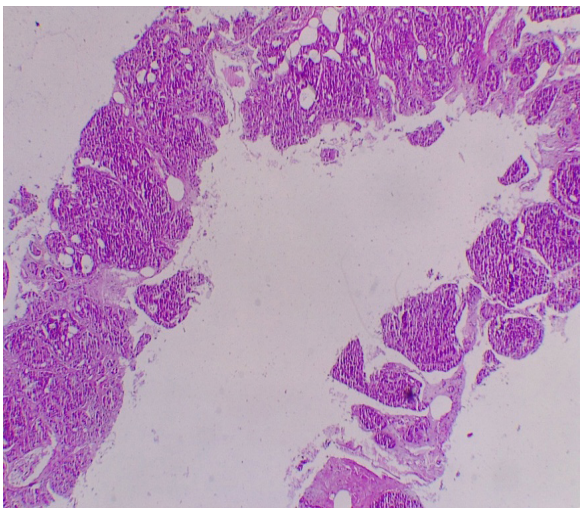
Prostate cancer gleason score 5, 10x.



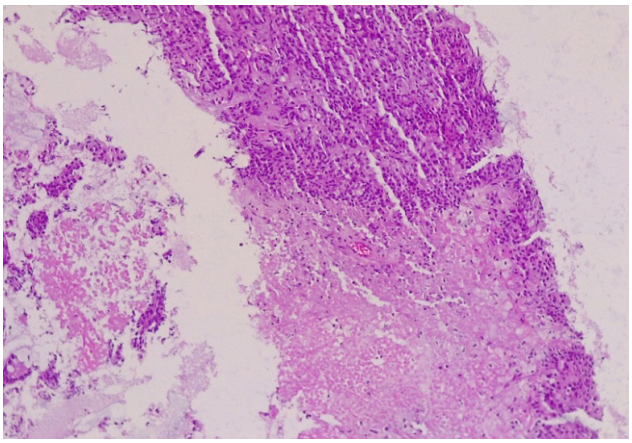
Prostate cancer gleason score 6, 10x



Prostate cancer gleason score 7, 10x.



Prostate cancer gleason score 8, 10x



Prostate cancer gleason score 9.

Figure 2. H&E staining, Gleason grading. Low power light microscope.

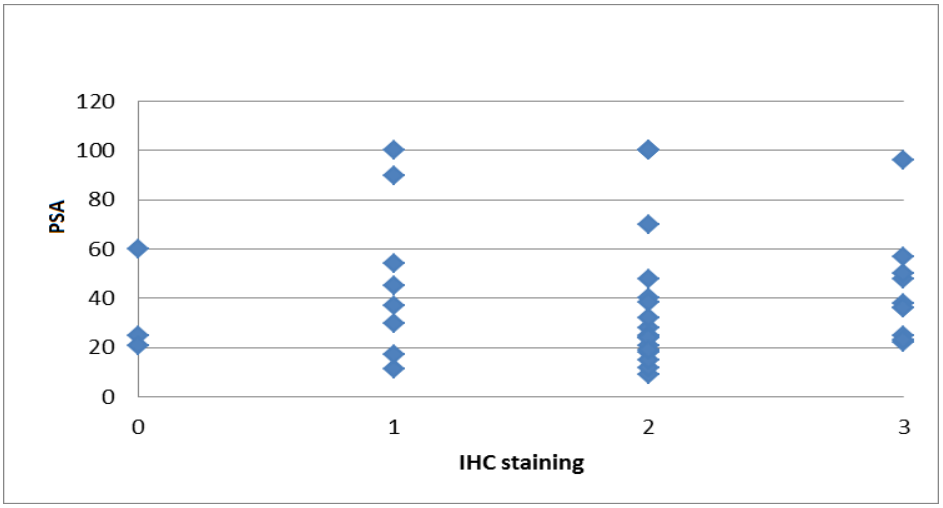


Figure 2. Scatter diagram shows individual value of CXCR4 staining level and PSA level for the patients.

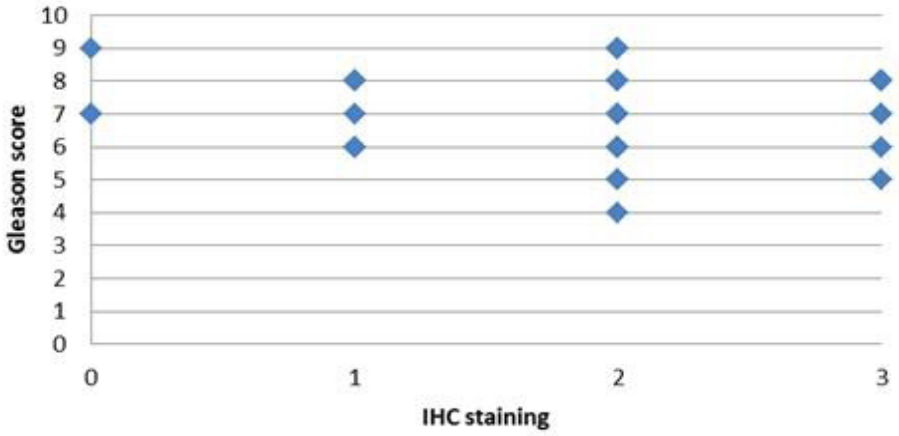


Figure 3. Scatter diagram to indicate individual value of CXCR4 staining level and Gleason score for each patient.

Table 2. Correlation between IHC staining score (CXCR4 staining level) and age of the patient with their PSA level and Gleason score.

	IHC 0	IHC1	IHC 2	IHC 3	P value
Age (Mean±SD)	73.33±8.38	73.12±9.77	70.84±6.31	72.3±8.13	0.874
PSA (Mean±SD)	35.33±21.45	48.04±32.21	43.34±33.12	43.1±22.02	0.938
Gleason score					
4	0	0	1	0	
5	0	0	2	1	0.206
6	0	4	6	1	
7	2	1	3	2	
8	0	3	3	6	
9	1	0	4	0	

DISCUSSION

Although world widely the level of PSA has been the only well accepted marker for prostate cancer diagnosis and prognosis, in the current study, we couldn't find any correlation between CXCR4 and PSA level, this result is consistent with the same finding by Zhong Lu et al [13]. The current study showed that CXCR4 presence and intensity is not related with tumor Gleason score, this finding was agreed by Seok et al [12] in their published series of 57 patients who had undergone operation for prostate cancer, Meena et al [14] also in a meta-analysis study with Joo Yong Lee et al revealed no relation between CXCR4 and Gleason score [15]. Although Seok et al [12] found no significant relation between the expression of CXCR4 and Gleason score, but they showed that local recurrence and distant metastasis were high in patients with high expression of CXCR4. Eventually their study concludes that CXCR4 expression can be used as a useful prognostic tool in cases of prostate cancer. The reverse was found in the study of Zhong Lu et al [13], they reported no significant association of CXCR4 expression and distant metastasis. The negative result in the current study between CXCR4 and clinic pathological parameters, might be related to small sample size and also missing some other more significant parameters such as tumor staging which was already missed or not registered during our work.

The current study showed no relation with a non-significant P value of 0.874 between the ages of the patients with prostate carcinoma and the nuclear score of CXCR4 IHC staining. This is consistent with the results reported by Seok et al [12] and Meena et al [14].

Although the current study involves a small number of cases, but it is the first type of work done on Iraqi patients to compare with previous publications.

There are few but crucial limitations for this study. First of all, the sample size is small which impacts on the level of significance. Second, Owing to the retrospective nature of the study, some pivotal role is missing like cancer staging and details of the participants. The study is a single center-based study which is more vulnerable to bias.

In conclusions, the high CXCR4 expression couldn't be related to the clinical parameters such as pretreatment PSA level, the Gleason core, and the age of the patients. However further studies with larger sample size is recommended to confirm the clinical significance of CXCR4 expression on patients with prostate cancer.

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