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Immunohistochemical Detection of Interleukin-6 in Iraqi Patients with Prostate Cancer

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Abstract:

Background: Prostate cancer is the second cancer causing death in men, after lung cancer, many etiological agents are proposed to play a role in its pathogenicity, one of these factors iscytokines.

Objective: To evaluate the immunohistochemical expression of IL6 and to establish the correlation between expression of IL6 and prostate cancer progression.

Methods: A case control study of 120 paraffin embedded prostatic tissue blocks, 50 cases were prostatic carcinoma (PCa), 50 cases were benign prostatic hyperplasia (BPH) and 20 cases were normal prostatic tissue. Sections from each block were prepared for immunohistochemical staining of IL6.

Results: 39 (78.0%) out of 50 prostatic carcinoma (PCa) cases demonstrated positive expression for IL6 specific antibody, the majority of PCa (56.4%) presented moderate positivity (++), (25.6%) showed intense positivity (+++) and (18.0%) showed weak positivity (+) against 11(22.0%) which offered negativism. While only 8 (16.0%) out of 50 benign prostatic hyperplasia (BPH) exhibited positive outcome when stained with this marker, comparing to 20 normal prostatic tissue (NPT) samples which revealed two positivity for IL6 antibodies.

Conclusion: Significant differences between expression of IL6 and histological type of prostatic lesions, between prostatic carcinoma, BPH and normal prostatic tissue, which may have potential to evolve to malignancy.

Keywords: Prostatic carcinoma, Interleukin 6, BPH, Immunohistochemistry.

1. INTRODUCTION:

Prostatic disease is a prevalent disorder worldwide and has a serious impairment in elderly men, especially lower signs of the urinary tract in the form of urinary retention, drop-out, hesitation and other disorders (1). After 40 years of age, prostatic glandular tissue begins to proliferate in 50% of males and 80% by 70 years of age (2). The proliferating prostatic tissue compresses the prostatic urethra, which contributes to substantial urinary impairment. Benign prostatic hyperplasia (BPH) is a disorder called (3). Prostate cancer is a prevalent disease in older men around the world and is the fifth-largest strong, non-cutaneous, non-hematological cancer in Iraq (4), and can be slow-growing and diagnosed in asymptomatic patients. Most prostate malignancies originate from glandular tissue, so adenocarcinoma is the most common form (5). Approximately 70% of prostate cancer rises from the peripheral zone, 15-20% and 10-15% in the central and

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transitional zones, respectively (6,7). Most prostatic carcinomas are multifocal, with several zones involved (8). The majority of patients with prostatic carcinoma are detected by asymptomatic male screening, prostatic specific antigen (PSA) serum level assessment, and digital rectal inspection (9). Moreover, when surgically extracted to alleviate obstructive urinary symptoms of BPH, prostatic cancer may be an unintentional pathological finding (10). Chronic inflammation allows cytokines and other inflammatory factors to be released, both from inflammatory cells and from the hypoxic epithelium of the prostate (11). Such cytokines interfere with stromal cells and cause further damage to the tissue (12).

Interleukin-6 (IL-6) is a versatile cytokine in human malignancies that is involved in tumor formation, invasion, and metastasis (13). A significant function of IL-6 has been suggested in the initiation of tumors and the progression of a number of cancers. Nguyen, et al., for example, indicated that IL-6 is an important modifier in the establishment of prostate tumor origin, tumor formation, metastasis, and chemotherapy resistance (14).

The goal of this study is to evaluate the immunoexpression of Interleukin-6 in benign, malignant prostatic lesions and normal prostate tissues and to distinguish the relationship between serum tPSA and IL6 immunoexpression and Gleason grade levels and the pathology of prostatic carcinoma cases.

2. MATERIALS AND METHODS:

2.1. Patient Group and Sample Collection:

In this case control study a total of (120) patients with prostate lesions consist of (50) patient with prostate cancer and (50) patient with benign prostate hyperplasia (BPH) as well as (20) apparently normal prostate tissue autopsies which were collected from the archives of Forensic Medicine Institute/ Baghdad and used as prostate healthy control groups. All the clinicopathological parameters, which included age of the patient, preoperative total serum PSA, histopathological type and Gleason grade for cases of prostatic carcinoma, were obtained from patient`s admission case sheets and pathological reports, Following trimming process, a consultant pathologist reexamined all these prostate tissues to further confirm the diagnosis. One section was mounted on ordinary glass slide and stained with hematoxyline and eosin, while another slide was mounted on charged slide to be used forimmunohistochemical study. The patients visited urological surgery unit in Ghazi al-Hariri Surgical Specialities Hospital in Baghdad province; and Al-Sader Teaching Hospital in Najaf province during the period (December, 2019 to August, 2020).

2.2. Inclusion and Exclusion Criteria:

The included criteria of the patients group involved in this study comprised any patient who had recent histologically diagnosed with prostate cancer and BPH. The healthy subjects were apparently seemed healthy and otherwise had no acute or chronic health problem.

2.3. Sample Processing for the IHC StainingTechnique:

1. The thickness of tissue section was 4 μm takenfrom blocks of paraffin embedded tissue on positivecharge slides when the cutting by microtome.
2. Incubations specimen on chargeable slide (slidewith tissue) for at least 2 hours at 58- 60 °C in theoven.

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3. At deparaffinization steps, 3 containers with Xyleneon which the tissues/slides immersed for 2 minutesfor each containers respectively.
4. Then the tissues/slides dipped in 3 jars with alcohol(ethanol), the first jars with 30% alcohol, the secondjars with 70% alcohol and the third jars with 100%alcohol, for 2 minutes at each jar respectively, this ishydration step.
5. The last steps, subjected tissues/slides in containerwith Immuno DNA Retriever Citrate and then thisRetrieval container with slide putin water bath set at 95 - 99°C for 60 minutes, then washed the tissues/slides 5 time with wash buffer
6. Then the immunohistochemical staining techniquewas applied (Bio SB- USA).

2.3.1. The Polyclonal antibodies used in the IHC staining procedure:

- **IL6 Polyclonal Antibody:** is a rabbit **polyclonal** IgG antibody intended to react against cytoplasmic antigen Interleukin-6 (IL6). It was derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing bovine serum albumin (BSA) and sodium azide as a preservative chemical compounds (BioSB).

2.4. IL-6 Scoring System:

Cells with visible brown particles in the cytoplasm were taken as positive. The immunohistochemical results were determined according to Zeng, et al., depending on the intensity of IL-6 staining (weak=1, intense=2), and the percentage of positive tumor cells (0%=0, 1%-50%=1, 51%-75%=2, >75%=3). The eventual score of each sample was determined by multiplying intensity by percentage score (18), and the tumors were ultimately categorized negative expression: score=0; low expression: score ≤ 3; or high expression: score >3.

2.5. Statistical Analysis:

Chi –square test and T- test were used to detect the significance between variables of our study. All the statistical analysis was done by SPSS program (Version–17) & P value was considered significant when $p < 0.05$.

3. RESULTS:

3.1. Distribution of Patients with Prostate Carcinoma, Prostate Benign and Control according to their Age:

The archival specimens collected in this study were related to prostate cancer patients whom ages were ranged from (45-85) years and the mean age of those prostate cancer patients was (65.22 ± 5.94) years, the mean age of patients with prostate benign was (65.16± 6.08) years and whom age ranged from (40-85) years, while the mean age of apparently healthy individuals (control) was (60.35±10.58) years and their mean age was ranged from (40- 75) years and the statistical analysis shows significant differences ($P<0.05$) between age strata distribution of control and prostate carcinoma; and control and prostate benign. However there was non-significant difference between prostate carcinoma and prostate benign as shown in table (1).

Table 1: Distribution of Study Groups According to the Mean and Range of their Age(Years)

Studied Groups	No	Mean age/	Std.	Std.	Range	LSD test

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		Year	Deviation	Error	Min	Max	(P-value)
Prostate cancer	50	65.22	5.94	0.84	52	**75**	P1= 0.960 **NS**
benign prostatic (BPH)	50	65.16	6.08	0.86	55	81	P2= 0.017 **S**
Control	20	60.35	10.58	2.36	40	78	P3= 0.02 **S**

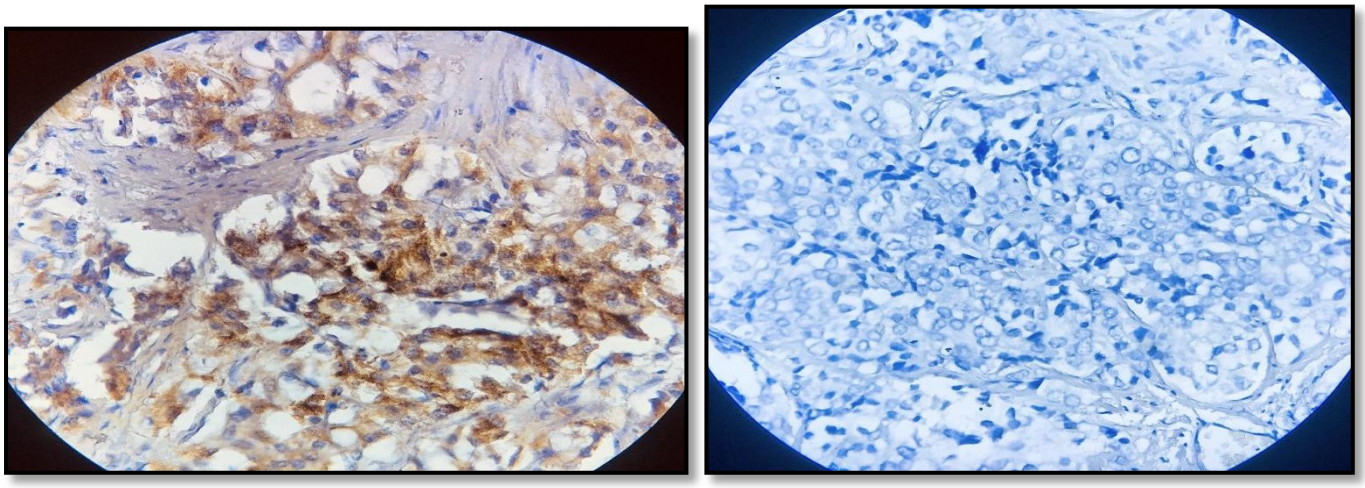
***P1:** Prostate cancer VS (BPH), **P2:** Prostate cancer VS Control; **P3:** BPH VS Control, **NS**: Not Significant, **S:** Significant

3.2. Immunohistochemical expression of IL6:

The evaluation of the immunolabeling for IL6 demonstrated that the majority of the BPH 6 (75.0%) presented weak positivity (+) and 2 (25.0%) presented moderate positivity (++) (Table 2). On other hand, the majority of PCa 22 (56.4%) presented moderate positivity (++), 10 (25.6%) showed intense positivity (+++) and 7 (18.0%) showed weak positivity (+) comparing to 20 normal prostatic tissue (NPT) samples which revealed two positivity for IL6 antibodies (Figure 4-1). There was statistically highly significant difference in expression of IL6 between cases of BPH and carcinoma prostate, indicated by P value of 0.001.

Table 4-2: Results of IL6 immunoexpression in Pca, BPH and NPT cases

IL6 expression	**BPH%**		**Pca%**		**NPT%**		***P*-value**
	No.	%	**No.**	%	**No.**	%	
Negative	42	84.0%	11	22.0%	18	90%	**P < 0.001** **¥** **HS**
	Positiveexpression		**Positiveexpression**		**Positiveexpression**		
Positive (+)	6	75.0%	7	18.0%	2	10%	
Positive (++)	2	25.0%	22	56.4%	0	0.0%	
Positive (+++)	0	0%	10	25.6%	0	0.0%	
Total	**50.0**		**50.0**		**20.0**		



n: number of cases; **¥:** Chi-square test; **HS:** Highly

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significant at $P \leq 0.0$

Figure1: Positive and Negative Expression of IL-6 by Immunohistochemistry onHuman Prostate Tissue(under 40X magnification power of light microscope). A. Positive Expression, B. Negative Expression

3.3. The Expression of IL-6 on Prostate Lesions and Age:

The results show in the prostate carcinoma, the most affected age stratum (70-79 years) was constituting (81.3%, 13) followed by the age stratum of (60-69 years) (80.0%, 8) and lowest affected group was the age stratum of more than 79 years and the age stratum of (60-69 years) which constituting (75.0%, 3 and 75.0%, 15 respectively), while in prostate benign the most affected age stratum of (<79 years) was constituting (25.0%, 1) and lowest affected group was age stratum (70-79 years) was constituting (18.7%, 3). The statistical analysis shows no significant differences (P>0.05) among age strata distribution of those studied groups prostate carcinoma and prostate benign with the positive IL6 cells staining as shown in the table (4-3).

Table 4-3: Statistical Analysis for the Distribution of Age Strata and Expression IL-6

Age group	IL-6 Expression in Pca		IL-6 Expression in BPH		*P*-value
	Positive *n* = 39	Negative *n* = 11	Positive *n* = 8	Negative *n* = 42	
<60, *n* (%)	8 (80.0%)	2 (20.0%)	2 (20.0%)	8 (80.0%)	**P1= 0.970 NS**
60-69, *n* (%)	15 (75.0%)	5 (25.0%)	3 (15.0%)	17 (85.0 %)	
70-79, n (%)	13 (81.3%)	3 (18.7%)	2 (12.5%)	14 (87.5%)	**P2=0.914 NS**
>79, *n* (%)	3 (75.0%)	1 (25.0%)	1 (25.0%)	3 (75.0%)	

P1: Pcapatients; **P2:** BPH patients

3.4. The Expression of IL-6 and Gleason grade, TNM Stages:

The results of IL6 expression and Gleason grade in prostate cancer show the expression was highest (92.9%) with high Gleason score Gs3 and lowest (50.0%) in low Gleason score Gs1 as shown in table (4-4). While, the results of IL6 expression and TNM staging in prostatic carcinoma, the expression was highest (100.0%) with high TNM staging (stage pT4) and lowest (50.0%) in low TNM staging (stage pT1) as shown in in table (4-4), according to these results there was no significant association (P>0.05) between IL-6 expressions and Gleason grade and there was a significant association (P<0.05) between IL-6 expressions TNM staging.

Table 4-4: IL-6 expressions and Gleason Grade and TNM Stages

Gleason Score	IL-6 Expression in Pca		*P*-value
	Positive *n* = 39	Negative *n* = 11	
Gs1 (3-5), *n* (%)	4 (50.0%)	4 (50.0%)	**0.065**

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Gs2 (6-7), *n* (%)	22 (78.6%)	6 (21.4%)	**¥ NS**
Gs3 (8-10), n (%)	13 (92.9%)	1 (7.1%)	
TNM Stage			
Stage T1, n (%)	4 (50.0%)	4 (50.0%)	**0.019 ¥ S**
Stage T2, n (%)	6 (60.0%)	4 (40.0%)	
Stage T3, n (%)	14 (82.3%)	3 (17.7%)	
Stage T4, n (%)	15 (100.0%)	0 (0.0%)	

n: number of cases; **¥:** Chi-square test; **NS:** not significant at $P \leq 0.05$, **S:** significant at $P \leq 0.05$

3.5. The Expression of IL-6 on Prostate Lesions and total PSA levels:

There was a significant increase in serum total PSA levels were found in Ca prostrate patients as compared to BPH patients ($p < 0.05$). As shown in table (4-6), no statistically significant correlation was found between serum total PSA levels and IL-6 positivity in prostate cancer and BPH.

Table 4-6: IL-6 expressions and PSA levels

PSA (ng/L)	**IL-6 Expression**		***P*-value**
	Positive n = 39	**Negative n = 11**	
Pca patients			
Range	7.0 – 23.0	7.0 – 15.0	**0.416 † NS**
Median (IQR)	11.0 (5.4)	10.8 (4.8)	
BPH patients			
	Positive n = 8	**Negative n = 42**	
Range	10.0 – 15.8	6.0 – 21.0	**0.056 † NS**
Median (IQR)	12.05 (3.95)	10.0 (4.55)	

****n*:** number of cases; **IQR:** inter-quartile range; **†:** Mann Whitney U test; **HS:** Highly significant at $P \leq 0.001$

4. DISCUSSION:

Chronic inflammation plays a significant role in the initiation and progression of the prostate lesion. Inflammation was believed to have a strong correlation to prostatitis, prostatic hyperplasia and prostate cancer. Inflammation will invite T cells, B cells and macrophages to the prostate glandular structures and stroma. After the initiation process, the dendritic cells will be activated and maintained the T cells responses within the prostate gland; this will cause a chronic and progressive pathological process that will eventually facilitate the progression of prostate hyperplasia or prostate cancer (15,16), table (1) showed that in both

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prostate carcinoma and benign, tumors have increased with the proceeding of age of patients and our results are closely agreed with the results obtained by (18), they revealed that the most common age of diagnosis in PC is between 45 and 75 years old. The present study is in agreement with study conducted by Singh *et al*, (18) who found that the incidence of prostate carcinoma increased as the age of men increased.

Immune cells infiltration will increase the secretion of a pro-inflammatory cytokine such as IL-2, IL-6, and IL-8. The activation of various cytokines will disrupt the balance of cell proliferation a d apoptosis. IL-6 is produced by various type of cells, including macrophages endothelial, and lymphocytes. IL-6 expression and can be detected both intracellular within cells cytoplasm as well on extracellular matrix.Higher expression of IL-6 was detected on a prostatecancer group with strong intensities. In the other hand,most of the prostate hyperplasia showed weakexpression of IL-6(17).

The present study showed a similar result with some other study. Engelhard *et al.,* (20) found that the expression of IL-6 in prostate cancer is significantly higher compared to prostate hyperplasia. We also observed a variation of IL-6 expression among prostate cancer; 7 of the samples exhibit a high IL-6 expression of IRS score 9 or above. The strong expression in some of prostate cancer samples raises the question of whether this finding has any relation to the biological behavior of cancer cells. Duscharla*et al.,* (21) reported that a high serum level of IL-6 is related to the bone metastasis of prostate cancer.

IL-6 secreted by immune cells infiltrate will be captured by IL-6-R, this will activate JAK, STAT3 and MAPK pathway, these, in turn, will induce cell proliferation through androgen receptor induction angiogenesis and facilitates metastasis. IL-6 are also known to induce intra-prostatic testosterone through activation of steroidogenic enzymes. Iliopoulos *et al.,* (22) suggested the correlationbetween inflammation, IL-6 activation, STAT1, PI3K,and NFkB in the pathogenesis of prostate cancer.Another study also confirmed that the increase of IL-6was correlated to the prognosis and showed anegative relation to the survival rate. Based on theabove results, the present study supports the theorythat IL-6 plays a role significant role in thepathogenesis and progression of prostate cancer (22).

However, in the present study, the expression of IL-6 was not correlated to the Gleason score significantly. Our study employed immunohistochemistry to detect the expression of IL-6 in prostate tissues. The nature of IL-6 as a soluble cytokine could sometimes be difficult to be measured quantitatively by immunohistochemistry (23).

In summary, the present study reveals the differential expression of IL-6 between prostate hyperplasia and prostate cancer. The IL-6 showed a higher expression in prostate cancer cells compared to prostate hyperplasia and normal prostate tissues. Further study is required to investigate the function of each cytokine in the pathogenesis of prostate lesion.

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