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Study the Correlation between Immunohistochemical Expression of TNFα and IFNɤ with Development of Prostate Cancer and BPH

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Abstract:

Background: Prostate diseases are the prevalent age-related disease of the male. Chronic inflammation strongly associated with both benign and malignant conditions of the prostate that leads to accumulation of immunocompetent cells which secret various cytokines that provide the suitable environment for various disease progression specially cancer

Objective: Evaluation of TNFα and IFNɤ immunoreactivity in prostatic carcinoma and benign prostatic hyperplasia (BPH) and to correlate this expression with clinicopathological parameters.

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 TNFα and IFNɤ.

Results: Negative expression of TNFα were observed in 18 (36.0%) out of 50 cases of BPH and positive expression were observed in 32 (64.0%) out of 50 cases of BPH, While the Pca cases revealed positive expression in 44 (88.6%) and negative expression were observed in 6 (12.0%). The results showed that 40 cases (80.0%) of BPH showed negative expression of IFNɤ, and 10 cases (20.0%) showed positive expression.On other side, the results showed that 26 cases (52.0%) of Pca showed negative expression of IFNɤ, and 24 cases (48.0%) showed positive expression.

Conclusion: This study concluded that there were differences in expression of both TNFα and IFNɤ between prostate hyperplasia and prostate cancer tissue by immunohistochemistry.

Keywords: Prostatic carcinoma, TNFα, IFNɤ, BPH, Immunohistochemistry.

1. INTRODUCTION:

Carcinoma of the prostate is an expanding risk throughout the world. It has the highest incidence of cancer among men and is the second cause of cancer-related death in men in the United States. However, it has variable mortality rates and incidence in the world (1). In Iraq, the incidence of prostatic carcinoma was 3.5% of all newly diagnosed cases of cancer in males (2).Prostate size enlargement is the outcome of an imbalance between apoptotic and proliferative activities, development of PCa or BPH is a complex process results from combination of molecular genetics and environmental factors. Age, family history, gene changes, sexually transmitted infections, smoking, chemical exposure, diabetes, heart diseases and lifestyle is important risk factors participate in the evolution of prostate infirmity (3,4).

In spite of researches over the years, the exact mechanisms underlying the development and progression of prostatic carcinoma are not obvious. The growth, development, and differentiation of the prostate are

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regulated by growth factors, steroid hormones particularly androgen, and cytokines (5).Cytokines are soluble proteins found in the prostate secretion fluid and serum of patients, which pick serious role in the inflammation, regulate growth, differentiation, activation and communication between immune cells (6). Each cytokine binds to specific surfacereceptor followed by subsequent cascades ofintracellular signaling that altered cell function,up regulation of several genes and theirtranscription factors result in the production ofother cytokines (7).One cytokine commonly found in the tumor microenvironment is TNF-α. Studies show that TNF-α is one of the pro-inflammatory cytokines with contradictory role in tumor process. It has anti-tumor properties, induces cancer cell apoptosis due to the sustained JNK (c-jun–NH2–kinases) activation, and as well, it has tumorigenic properties, linking inflammation with carcinogenesis through regulation of expression of cytokines promotes cell proliferation and tumor growth (8).

In prostate tumors, pro-inflammatory cytokines, (Tumor Necrosis Factor-Alpha (TNF-a) and Interferon gamma (IFN-g)), that are secreted by tumor-infiltrating lymphocytes (TILs) play major roles in the immune response against cancer cells. Thus, TNF-α induces cancer cell death as well as, can promote tumor cell survival, proliferation, migration and angiogenesis. It is therefore important to find out how to selectively inhibit the tumorigenic properties of TNF-α, and saving its antitumor properties (9). IFN-γ and TNF-α are typically produced in the context of ongoing immune responses against infectious and tumor challenges. However, TNF-α has also been proposed as PCa marker (10). Regarding IFN-γ, PCa cells have been shown to be poorly sensitive to its cytotoxic effects. Furthermore, it has been suggested that IFN-γ might induce immune-suppressive effects in PCa (11).

In this study, we evaluate the immunohistochemical expression of TNFα and IFNɤ in benign and malignant prostate and investigatethe association with pathological grading and clinical staging in Iraqi patients with prostatic carcinoma.

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. The prostate carcinoma samples include 8 cases were obtained from needle biopsies, 30 cases were from Transurethral Resection of the Prostate (TURP), and 12 were obtained from open prostatectomy specimens. 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).

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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.
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:

- **TNF alpha Polyclonal Antibody:** is a rabbit polyclonalIgG antibody intended to react against cytoplasmic antigen tumor necrosis factor alpha (TNFα). 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).
- **IFNG Polyclonal Antibody:** is a rabbit polyclonalIgG antibody intended to react against cytoplasmic antigen interferon gamma (IFNγ). 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. TNFα and IFNɣ Scoring System:

Cells with visible brown particles in the cytoplasm were taken as positive. Estimating the number of cells that give brown cytoplasmic staining (positive) under a light microscope. The intensity of immunohistochemical staining was estimated in 10 fields (X40 magnification). The total number of cells in each field wascounted. The total score for staining was divided by the number of all cells per field in 10 fields, so the positivity stained cells percentage (in the 10 fields) was estimated for each case by holding the mean of the percentage of the cells with a positivestain in the 10 fields. The eventual score of each sample was determined by multiplying intensity by percentage score (12), and the tumors were ultimately categorized as follows; 1+ = positive cell rate <25%; 2+ = staining in at least 25–50%of tumor cells; 3+ = strong, staining in 51–75% of invasive carcinoma cells; 4+ = staining in >75% of tumor cells.

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2.5. Statistical Analysis:

Were summarized, presented and analyzed using statistical package for social science (SPSS version 24) and Microsoft Office Excel 2013. Numeric data were presented as mean, standard deviation, while nominal data were expressed as number and percentage. Independent sample T test was used to compare mean value between two parametric groups while. Chi-square test was used to compare between nominal data. Correlation coefficient was estimated by spearman correlation.

RESULTS:

2.6. 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 withprostate 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 highly significant differences (P<0.01) between age strata distribution ofcontrol 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

Studied Groups	No	Mean age/ Year	Std. Deviation	Std. Error	Range		LSD test (P-value)
					Min	Max	
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

2.7. Morphological evaluation results:

Carcinoma prostate patients were categorized according to Gleason's score (combined Gleason's grade). Gleason's score of 6 was the commonest pattern observed in 16 (32.0%) cases, followed by Gleason's score of 7 in 12 (24.0%) cases and Gleason's score of 8 in 9 (18.0%) cases. Gleason's score of 4, 5, 9 were seen in 3 (6.0%), 5 (10%), 4(8.0%) cases each and Gleason's score of 10 was seen in only one (2%) case. In these study, Based on tumor differentiation 4 cases (8.0%) were well differentiated with a score of 2–4 while 31 (62.0%) were moderately differentiated with a score of 5–7 and 15 cases (30%) were poorly differentiated with a score of 8–10.

According to tumor, node, and metastasis (TNM) staging system, the pathological stage in prostatic carcinoma: 8 (16.0%) tumors were stage I (pT1), 10 (20.0%) tumors were stage II (pT2), 17 (34.0%) were stage III (pT3), and 15 (30.0%) were stage IV (pT4).

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3.8. Immunohistochemical expression of TNFα:

TNF-α is expressed in the cytoplasm of the tumor cells, negative expression were observed in 18 (36.0%) out of 50 cases of BPH and positive expression were observed in 32 (64.0%) out of 50 cases of BPH (6 cases (21.8%) were strong positive, 14 cases (43.8%) were moderate positive, and 11 cases (34.4%) were weak positive) (Table 2).While the Pca cases revealed positive expression in 44 (88.6%), the majority of PCa 18 (41.0%) presented moderate positivity (++), 12 (27.2%) showed intense positivity (+++) and 14 (31.8%) showed weak positivity (+)comparing to 20 normal prostatic tissue samples which revealed four positivity for TNFα antibodies (Figure 1). There was statistically highly significant difference in expression of TNFαbetween cases of BPH and carcinoma prostate, indicated by P value of 0.001.

Table 2: Results of TNFα immunoexpression in Pca, BPH and NPT cases

TNFα expression	BPH%		Pca%		NPT%		*P*-value
	No.	%	No.	%	No.	%	
Negative	18	36.0%	6	12.0%	16	80.0%	HS P < 0.001 ¥
	Positiveexpression		Positiveexpression		Positiveexpression		
Positive (+)	11	34.4%	14	31.8%	2	50.0%	
Positive (++)	14	43.8%	18	41.0%	2	50.0%	
Positive (+++)	7	21.8%	12	27.2%	0	0.0%	
Total	50.0		50.0		20.0		

***n*:** number of cases; **¥:** Chi-square test; **HS:**Highly significant at $P \leq 0.001$

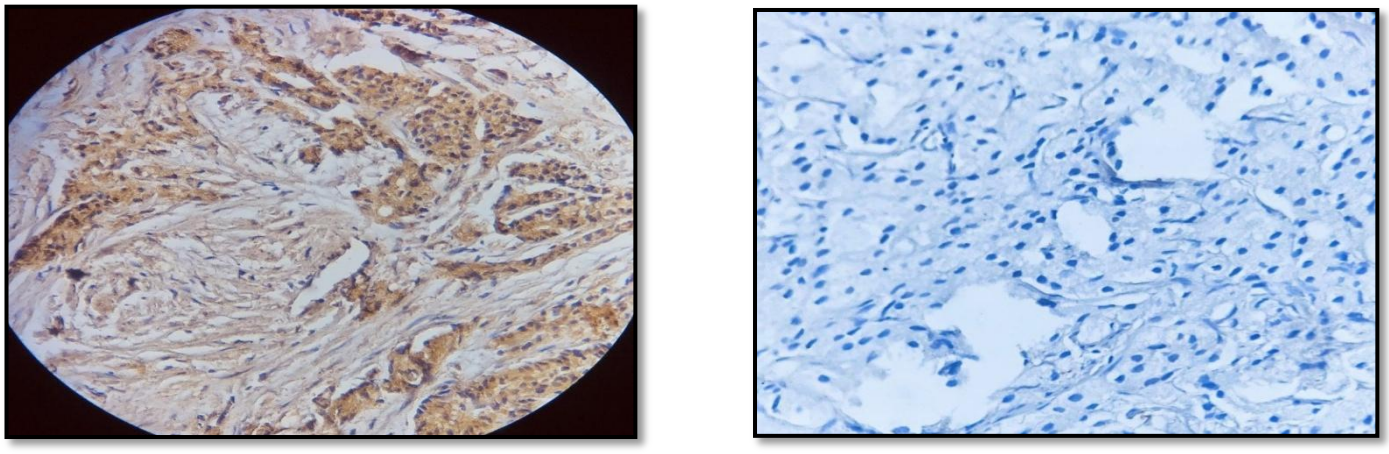

Figure 1: Positive and Negative Expression of TNFα by Immunohistochemistry onHuman Prostate Tissue (under 40X magnification power of light microscope). A. Positive Expression, B. Negative Expression

3.8.1. The Expression of TNFα on Prostate Lesions and Age:

The results showin the prostate carcinoma, the mostaffected age stratum (>79 years)was constituting (100.0%, 4)followed by the age stratum of (70-79 years) and (60-69 years)were constituting (93.8%, 15;

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90.0%, 18)and lowest affected group was the age stratum of lessthan 60 years which constituting (70.0%, 7).while inprostate benign the most affected age stratum of (60-69 years) was constituting (63.6%, 14) and lowest affected group was the age stratum of more than 79 years (50.0%, 2). The statistical analysis shows no significant differences (P>0.05) among age strata distribution of those studied groups prostate carcinoma and prostate benign as shown in the Table (3).

Table 3: Statistical Analysis for the Distribution of Age According to Expression TNFα

Age group	TNFα Expression in Pca		TNFα Expression in BPH		*P-value*
	Positive *n* = 44	Negative *n* = 6	Positive *n* = 32	Negative *n* = 18	
<60, *n* (%)	7 (70.0%)	3 (30.0%)	6 (60.0%)	4 (40.0 %)	**P1= 0.242 NS**
60-69, *n* (%)	18 (90.0%)	2 (10.0%)	14 (63.6%)	8 (33.4%)	
70-79, n (%)	15 (93.8%)	1 (6.2%)	10 (62.5%)	6 (37.5%)	**P2=0.658 NS**
>79, *n* (%)	4 (100.0%)	0 (0 %)	2 (50.0%)	2 (50.0%)	

P1: Pca patients; **P2:** BPH patients, **NS**: Not Significant

3.8.2.The Expression of TNFα and Gleason grade, Pathological Stages:

Regarding TNFα immunohistochemical expression and Gleason scoring, the expression was highest (92.8%) in prostatic carcinoma with high Gleason score Gs2 and Gs3 and lowest (62.5%) in low Gleason score Gs1 as shown in table (4).

Regarding TNFα immunohistochemical expression and TNM staging, the expression was highest (100.0%) in prostatic carcinoma with high TNM staging (stage pT4) and lowest (75.0%) in low TNM staging (stage pT1) as shown in in table (4).

Table 4: TNFα expressions and Gleason Grade and TNM Stages

Gleason Score	TNFα Expression in Pca		*P*-value
	Positive *n* = 44	Negative *n* = 6	
Gs1 (3-5), *n* (%)	5 (62.5%)	3 (37.5%)	**0.053 ¥ NS**
Gs2 (6-7), *n* (%)	26 (92.8%)	2 (7.2%)	
Gs3 (8-10), *n* (%)	13 (92.8%)	1 (7.2%)	
TNM Stage			
Stage T1, *n* (%)	6 (75.0%)	2 (25.0%)	**0.269 ¥ NS**
Stage T2, *n* (%)	8 (80.0%)	2 (20.0%)	
Stage T3, *n* (%)	15 (88.2%)	2 (11.8%)	
Stage T4, *n* (%)	15 (100.0%)	0 (0.0%)	

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

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3.8.3. The Expression of TNFα on Prostate Lesions and total PSA levels:

As shown in table (5), there was no significant association between TNFαpositivity and serum total PSA levels in prostate cancer and BPH and figure (2).

Table 5: TNFα expressions and PSA levels in Pca and BPH

PSA (ng/L)	TNFα Expression		*P*-value
	Positive n = 44	**Negative n = 6**	
Pca patients			
Range	6.0 – 23.0	8.5 – 13.6	**0.395 † NS**
Median (IQR)	10.6 (6.67)	9.55 (3.15)	
BPH patients			
	Positive n = 32	**Negative n = 18**	
Range	5.0 – 21.0	5.0 – 15.2	**0.279 † NS**
Median (IQR)	9.75 (4.62)	8.9 (2.55)	

***n*:** number of cases; **IQR:** inter-quartile range; **†:** Mann Whitney U test; **NS:** not significant at $P \leq 0.05$

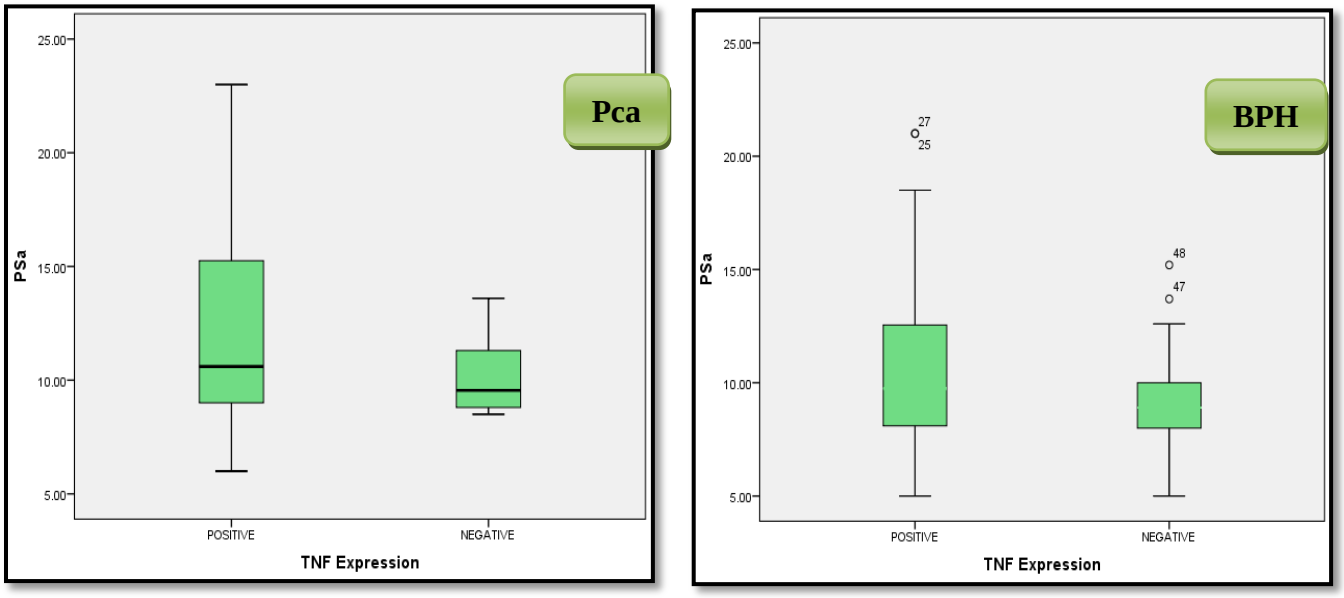

Figure 2: TNFα expressions and PSA levels in Pca and BPH

3.9. Immunohistochemical expression of IFNɣ:

The results showed that 40 cases (80.0%) of BPH showed negative expression, and 10 cases (20.0%) showed positive expression (1 cases (10.0%) were strong positive, 4 cases (40.0%) were moderate positive, and 5 cases (50.0%)) were weak positive) (Table 6). On other side, The results showed that 26 cases (52.0%) of Pca showed negative expression, and 24 cases (48.0%) showed positive expression, the majority of PCa 10 (41.7%) presented intense positivity, 9 cases (37.5%) were moderate positive, and 5 cases (20.8%)

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showed weak positivity comparing to 20 normal prostatic tissue samples which revealed total negativity for IFNɣ antibodies (Figure 3).There was statistically a highly significant difference in expression of IFNɣbetween cases of BPH and carcinoma prostate, indicated by P value of 0.001.

Table 6: Results of IFNɣ immunoexpression in Pca, BPH and NPT cases

IFNɣexpression	BPH%		Pca%		NPT%		*P*-value
	No.	%	No.	%	No.	%	
Negative	40	80.0%	26	52.0%	20	100%	P=0.001 ¥ HS
	Positiveexpression		**Positiveexpression**		**Positiveexpression**		
Positive (+)	5	50.0%	5	20.8%	0	0.0%	
Positive (++)	4	40.0%	9	37.5%	0	0.0%	
Positive (+++)	1	10.0%	10	41.7%	0	0.0%	
Total	**50.0**		**50.0**		**20**		

***n*:** number of cases; **¥:** Chi-square test; **HS:** Highly significant at $P \leq 0.001$

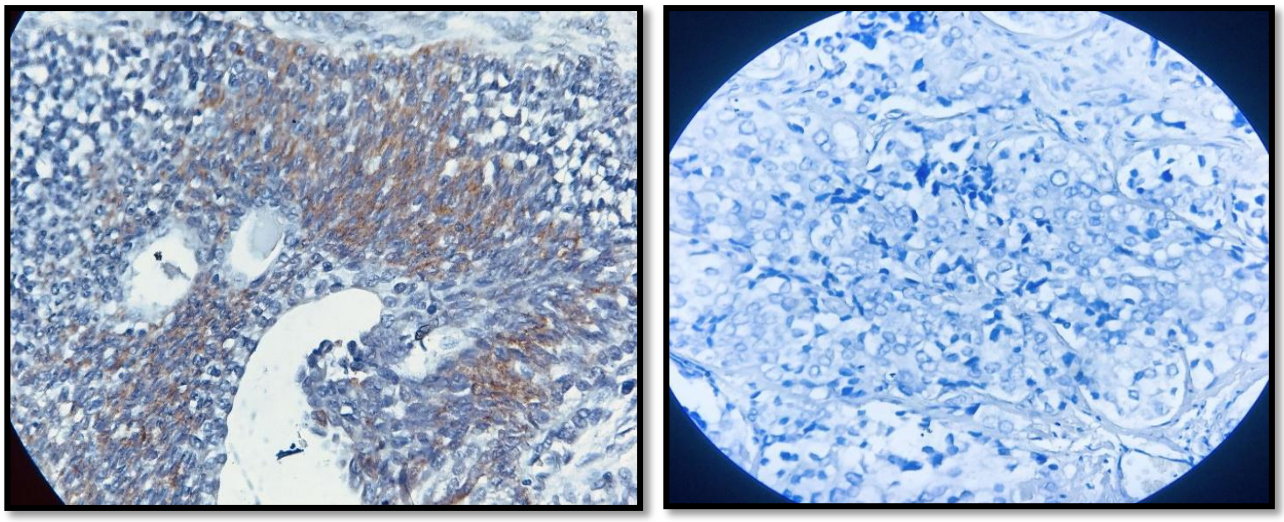

Figure 3: Positive and Negative Expression of IFNɣby Immunohistochemistry onHumanProstate Tissue (under 40X magnification power of light microscope). A. Positive Expression, B. Negative Expression

3.9.1. The Expression of IFNɣon Prostate Lesions and Age:

The results show in the prostate carcinoma, the most affected age stratum was less than 60 years constituting (90.0%, 9)and lowest affected group was the age stratum of (60-69 years) which constituting (35.0%, 7), while in prostate benign the most affected age stratum of (>79 years) was constituting (25.0%, 1) and lowest affected group was the age stratum of (60-69 years) (18.8%, 3). The statistical analysis shows that there are significant differences (P>0.05) among age strata distribution of prostate carcinoma groups and expression ofINFɣas shown in the table (7).

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Table 7: Statistical Analysis for the Distribution of Age According to Expression TNFα

Age group	INFɤ Expression in Pca		INFɤ Expression in BPH		*P*
	Positive *n* = 24	Negative *n* = 26	Positive *n* = 10	Negative *n* = 40	
<60, *n* (%)	9 (90.0%)	1 (10.0%)	2 (20.0%)	8 (80.0%)	**P1= 0.028 S**
60-69, *n* (%)	7 (35.0%)	13 (65.0%)	4 (20.0%)	16 (80.0%)	
70-79, n (%)	6 (37.5%)	10 (62.5%)	3 (18.8%)	13 (81.2%)	**P2=0.994 NS**
>79, *n* (%)	2 (50.0%)	2 (50.0%)	1 (25.0%)	3 (75.0 %)	

P1: Pca patients; **P2:** BPH patients, **NS**: Not Significant, **S:** Significant

3.9.2. The Expression of IFNɤand Gleason grade, TNMStages:

Regarding to the results of IFNɤimmunohistochemical expression and Gleason scoring, the expression was highest (71.4%) in prostatic carcinoma with high Gleason score Gs3 and lowest (25.0%) in low Gleason score Gs1 as shown in table (8). On the other hand, the results of IFNɤimmunohistochemical expression and TNM staging, the expression was highest (66.6%) in prostatic carcinoma with high TNM staging (stage pT4) and lowest (25.0%) in low TNM staging (stage pT1) as shown in in table (3-4), according to these results there was no significant association (P>0.05) between TNFα expressions and Gleason grade and TNM staging.

Table 8: IFNɤexpressions and Gleason Grade and TNM Stages

Gleason Score	IFNɤExpression in Pca		*P*-value
	Positive *n* = 24	Negative *n* = 26	
Gs1 (3-5), *n* (%)	2 (25.0%)	6 (75.0%)	0.079 ¥:
Gs2 (6-7), *n* (%)	12 (42.8%)	16 (57.2%)	
Gs3 (8-10), n (%)	10 (71.4%)	4 (28.6%)	
TNM Stage			
Stage T1, n (%)	2 (25.0%)	6 (75.0%)	0.154 ¥
Stage T2, n (%)	3 (30.0%)	7 (70.0%)	
Stage T3, n (%)	9 (52.9%)	8 (47.1%)	
Stage T4, n (%)	10 (66.6%)	5 (33.4%)	

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

3.9.3. The Expression of IFNɤ on Prostate Lesions and total PSA levels:

As shown in table (9), there was no significant association between IFNɤpositivity and serum total PSA levels in prostate cancer, but there was a significant association between IFNɤpositivity and serum total

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PSA levels in BPH and figure (4).

Table 9: IFNɣ expressions and PSA levelsin Pca and BPH

PSA (ng/L)	IFNɣ Expression in Pca		*P*-value
	Positive n = 24	**Negative n = 26**	
Pca patients			
Range	8.1 – 21.0	7.0 – 21.0	**0.298 † NS**
Median (IQR)	11.15 (3.6)	10.3 (5.55)	
BPH patients			
	Positive n = 10	**Negative n = 40**	
Range	6.0 – 15.0	5.0 – 12.6	**0.028 † S**
Median (IQR)	9.4 (4.35)	8.5 (4.55)	

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

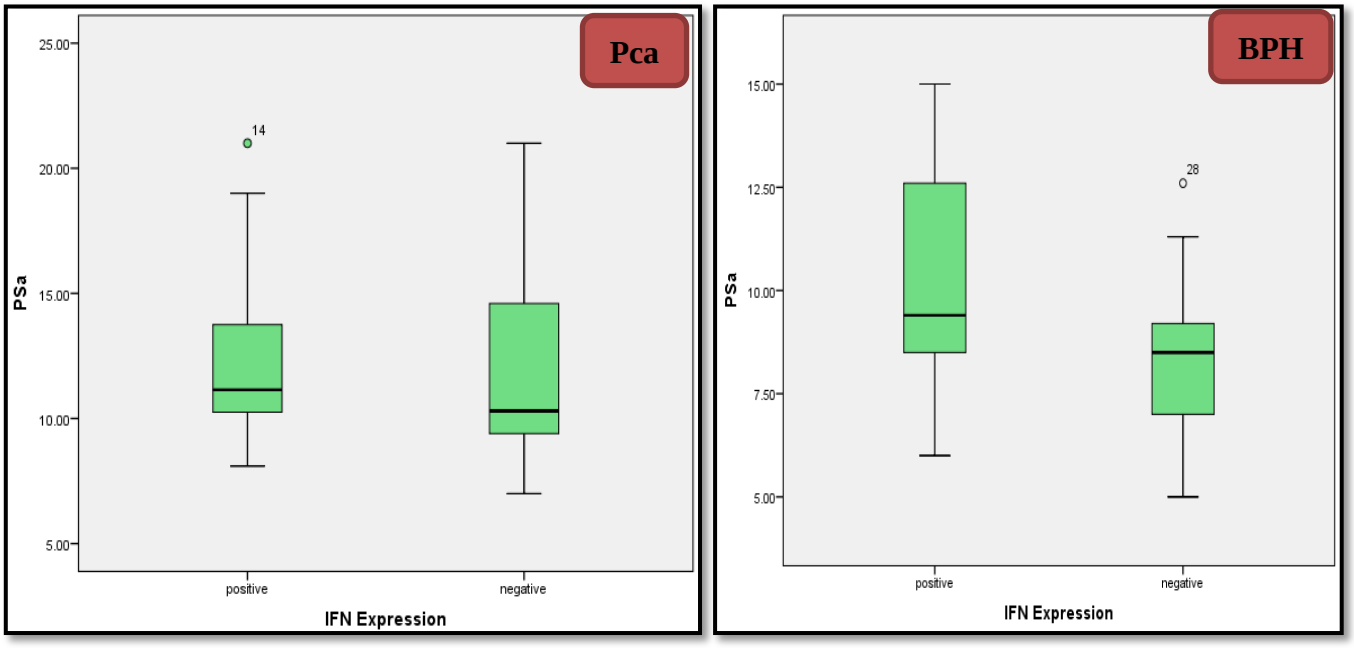

Figure 4: IFNɣ expressions and PSA levels in Pca and BPH

3. DISCUSSION:

Inflammation has now been recognized to contribute to proliferation, malignancy, angiogenesis,metastasis, modulation of adaptive immunity, and unresponsiveness to hormones and chemotherapeuticagents (13). Inflammation will invite T cells, B cells and macrophages to the prostate glandular structures and stroma.

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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 (14). Table (1) showed that in both 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*, (15) who found that the incidence of prostate carcinoma increased as the age of men increased.

The role of TNF-α in tumor activation and cancer progression is controversial. Tumor necrosis factor alpha (TNF-α), isolated 30 years ago, is an extraordinarily pleiotropic cytokine, that plays important roles in diverse cellular events, cell survival, proliferation, differentiation, and death, with a central role in immune homeostasis, inflammation, and host defense. TNF can induce apoptosis, necrosis, angiogenesis, immune cell activation, and cell migration. Although named for its antitumor properties, TNF has been implicated in tumor development and tumor progression. In regard to cancer, TNF could be an endogenous tumor promoter, through stimulation of cancer cells' growth, proliferation, invasion and metastasis, and tumor angiogenesis. On the other hand, TNF displays pro- and antitumoral effects (16). TNF-α is a proinflammatory cytokine, that is associated with cancer progression. There has been a study by (17), where TNF-α is associated with prostate cancer progression.

The present results revealed a statistically significant expression of TNF-α in malignant tumors in comparison with the benign tumors, positive expression of TNF-α is identified in most cases of prostate cancer (44, 88.0%), While 32 (64.0%) benign cases were positive for TNF-α. In this study, the expression of TNF-α among malignant tumors is slightly higher in that with higher grade compared to that of lower grade, lower stage, these results were agreed with results of Duarsa *et al.,* (18)and Gong *et al.,* (19).

Interferon γ is a cytokine whose biological activity is conventionally associated with cytostatic/cytotoxic and antitumor mechanisms during cell mediated adaptive immune response. The finding of these results reported significantly higher interferon-gamma expression in prostate cancer patients compared to controls group. The PCa patients with a Gleason score of 7-9 have the highest amount of IFN-Gamma. Men with advanced age of PCa were found to have high plasma concentrations of IFN- gamma in other comparable findings of (20). The expression of IFN Gamma among the BPE patients were less than those of the PCa patients. IFN-Gamma expression were significantly high within the various Gleason scores. The findings are similar to those in a study by Tazaki *et al*., (21) and Banzola *et al.,* (22) where IFN-Gamma levels were found to be higher than normal in cachexic prostate cancer patients. Interferon Gamma is a Th1 cytokine produced by phagocytic cells (monocytes, macrophages and neutrophils) and dendritic cells (DC), and activate natural killer (NK) cells (representative anticancer immune cells) leading to anticancer effects. IFN Gamma accordingly plays crucial anticancer effects *in vivo* and it is thought to suppress cancer development and metastasis (23). The data showed that IFN-Gamma also has potential as a biomarker for prostate cancer.

In summary, the present study reveals the differential expression ofTNFα and IFNɤ between prostate hyperplasia and prostate cancer. The TNFα and IFNɤ showed a higher expression in prostate cancer cells

compared to prostate hyperplasia. Further study is required to investigate the function of each cytokine in the pathogenesis of prostate lesion.

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