Immunohistochemical Assessment of Tumor Infiltrating T Cells and Serological Evaluation of Apoptotic Markers in Prostate Cancer Patients

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Abstract

PCa is abnormal transformed growth in glandular prostate tissue of male reproductive system. Occasionally, it is slow growth, but some progressed rapidly. This corss-sectional study aims to evaluate the differences in counts of tumor infiltrating immune cells (CD3 & CD8), and serum soluble Fas and Fas ligand concentrations between malignant and benign PCa. Total of (17) patients with PCa were included, their ages ranged from (38-88) years. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (November 2018 to October 2019). The samples comprise biopsy for estimation of tumor infiltrating CD3 & CD8 by IHC technique, and 3 mL of blood for sFas and sFasL evaluation by ELISA technique for each patient. The results showed the differences of CD3+ & CD8+ PILs counts between BPH and malignant PCa are non-significant (P value > 0.05), sFas concentrations of malignant PCa are significantly higher (P value < 0.05) than those of BPH, whereas no significant difference (P value > 0.05) in sFasL concentrations. It was concluded that prostate tumor type affects serum levels of sFas, but both CD3+ & CD8+ PILs rates and sFasL levels are not affected.

Keywords: *PCa, Malignant, BPH, TILs, sFas, sFasL; toxicity*

Introduction

Prostate cancer (PCa) is the abnormal growth of the prostate gland tissue, in the reproductive system of male. Most prostate tumors are slow growing; but, some develop rapidly [1]. Furthermore, Patients with metastatic prostate carcinomas have high incidence of bone lesions, leading to persistent prostate cancer-induced bone pain (PCIBP) [2]. Environmental factors account the highest percent of cancer such as tobacco use, obesity and nutrition, infections, ionizing and non-ionizing radiation, lack of physical activity, and pollution [3].

In Europe, in 2012 it was the third most diagnosed cancer after breast and colorectal at 417,000 cases [4]. The incidence and prevalence of prostate cancer vary in different parts of the world, with the highest in Australia, New Zealand, Northern & Western Europe, and North America and the lowest in South Asia [5].

Prostatic cancer is rarely symptomatic early in its course and therefore disease presentation often implies local extension or even metastatic disease [6]. It is very important to detect and diagnose prostate cancer in its earliest stages, often prior to the presentation of symptoms [6].

Infiltrating immune cells are of particular interest since there is evidence that inflammation plays a role in malignant transformation of multiple organs, including the prostate [7]. During prostate carcinogenesis, both innate immune cells such as macrophages, and adaptive immune cells (T and B cells) were reported to accumulate in the prostate [8]. However, their contribution to

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prostate cancer development is contradictive, since both promotion and inhibition of carcinogenesis has been described [9]. The study aimed to evaluate the differences in counts of tumor infiltrating immune cells (CD3 & CD8), and serum soluble Fas and Fas ligand concentrations between malignant and benign PCa.

Material & Methods

In this cross-sectional study, total of (17) patients with prostate cancers (PCa) were included. Their ages ranged from (38-88) years. Case information for each patient included: name, age, and the diagnosed tumor type were taken from the report of the diagnosis. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (November 2018 to October 2019).

Included & Excluded Criteria:

The included criteria comprised any patient who had recent histologically diagnosed with prostate tumors. Excluded criteria are comprised; any case that had normal histological results after suspicion of prostate tumor, any patient who took chemotherapy, and any case of retrospective prostate tumor.

Ethical Approval:

Agreement of all patients or their sons and/or first degree relative (father & mother) has been taken prior take any specimen. Moreover, the study design was approved by Research Ethical Committee in College of Medicine / Babylon University.

Samples:

Prostate biopsies were obtained from the urological surgery unit after patient admission. The biopsy was saved in plastic tube container with 10% neutral buffered formalin (NBF) before processing through paraffin embedding technique to create a formalin-fixed, paraffin-embedded (FFPE) blocks for immunohistochemical tests (CD3 & CD8). Also, three milliliter of blood for each patient was collected through vein puncture, put in sterile Gel tube. The collected blood samples were left in room temperature for 15-30 minutes to clot before they were centrifuged at 1000×g for15 min to remove the clot, and then the upper layer (serum) was separated by pipette, then put it in clean Eppendorf tube and stored at -20C° until using in serological tests (sFas & sFasL)**.**

Methods

Sample Processing for the IHC Staining Technique:

· By microtome which its blades are standardized at 4 μm, each block of FFPE tissue was sectioned into 4 μm thickness section (one section for each IHC marker), then the sectioned tissue was put in water bath (40-50) °C for seconds that allow the tissue to be relaxed, and allow the paraffin to stick to the glass. Then sections were mounted on positive charge slide.

· After that, mounted slides were incubated in oven (58-60) °C for at least two hours.

· The third step is deparaffinization; three containers filled with Xylene were prepared, then mounted slides were immersed on each container for 2 minutes, sequentially.

· The fourth step is hydration; three jars were prepared with ethanol (the first one with 30% ethanol, the second with 70% ethanol, and the third with 100% ethanol), then deparaffinized slides were immersed on each jar for 2 minutes started with the first one of 30% ethanol and continued upward.

· In the last step : the hydrated slides were subjected in Retrieval container with Immuno DNA Retriever Citrate, and with each other were put in water path (95-99)°C for 1 hour.

· Then staining by immunohistochemical technique was applied (Bio SB- USA).

Evaluation of CD3 and CD8:

Presence of brown colored reaction in the nucleus or cytoplasm was considered a positive reaction. The intensity of the immunostaining was determined by modified way through taking a pictures for three fields of each section through digital camera connected to conventional light microscope (20X power), and further image analysis was done with the Image J software (version 1.46r, National Institutes of Health, USA) for each picture to count (CD3 or CD8) cells, then the mean value was calculated that represent the cells number per field for each section [10, 11].

ELISA Technique:

The principle of sandwich ELISA technique has been applied for the assessment of serum soluble (Fas/CD95 & FasL/TNFSF6) according to instructions of company (Elabscience-USA).

Statistical Analysis

By using Software of Statistical Package for the Social Sciences (SPSS), version 26.0, to investigate the differences of study parameters between malignant and BPH. The differences between two groups were analyzed by Independent t-test. Statistically, it is considered a significant difference when P value <0.05.

Results

There are 17 subjects were diagnosed as prostate cancers (PCa). These cancers are subdivided into; 10 (59%) malignant prostate cancer (adenocarcinoma), and 7 (41%) benign prostatic hyperplasia (BPH).

Immune cells (CD3 & CD8):

As shown in Table (1), the findings showed that CD3+ prostate infiltrating lymphocytes (PILs) counts in BPH (143.8 cells/field ±41) are slightly lower than that of malignant (174 cells/field ±97), and statistically there is no significant (P value > 0.05) difference between them. Also, CD8+ PILs counts in malignant prostate cancer (88.4 cells/field ±35.8) do not significantly different (P value > 0.05) from that in BPH (83.9 cells/field ±38.6).

Table (1): Comparison of tumor infiltrating CD3+ and CD8+ T cells counts between Benign and Malignant prostate cancers.

Parameters	Benign	Malignant	P value
CD3 cells/field	143.8±41	174±97	0.395
CD8 cells/field	88.4±35.8	83.9±38.6	0.808

* represents a significant difference at p<0.05. Data are expressed as Mean±SD.

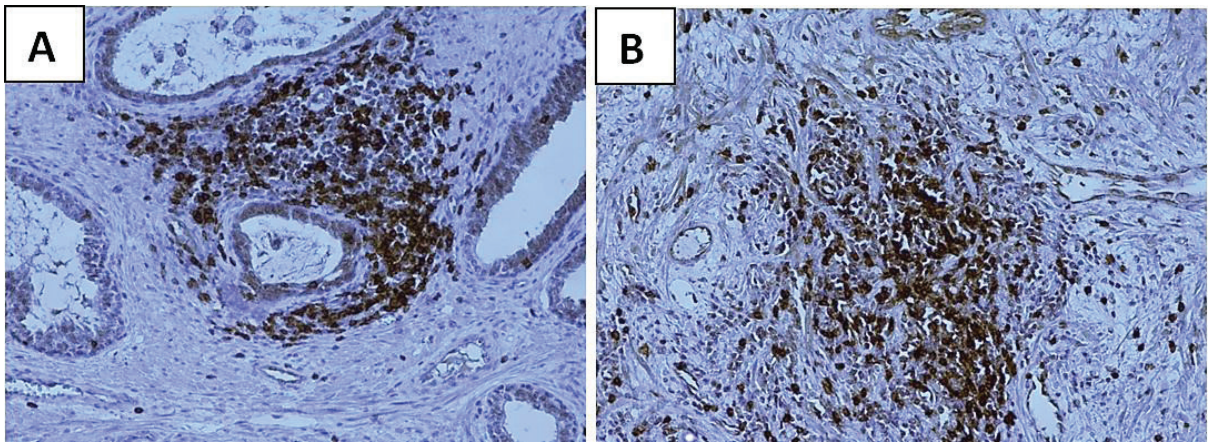

Figure (1): PCa slide with CD3 marker (20X Magnification Power): (A) CD3+ TILs in malignant PCa (B) CD3+ TILs in BPH.

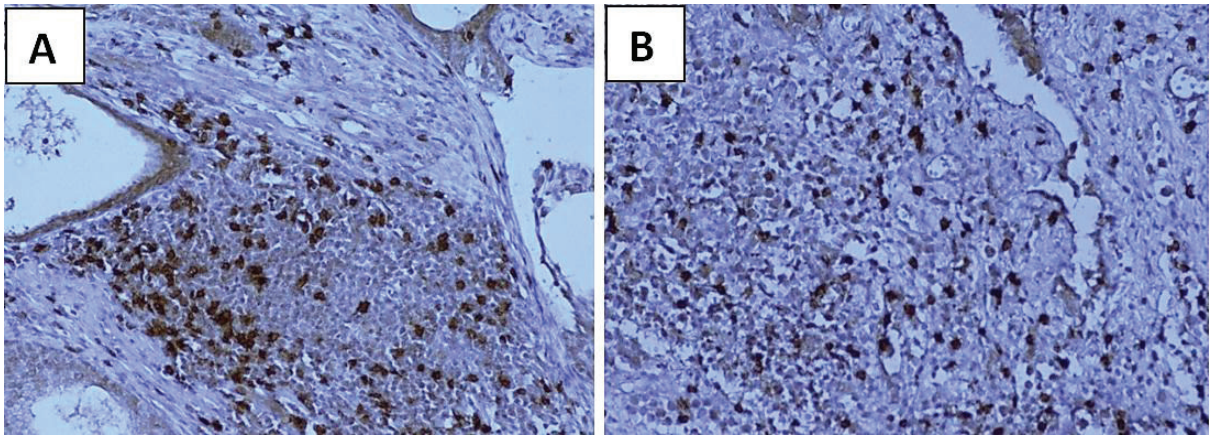

Figure (2): PCa slide with CD8 marker (20X Magnification Power): (A) CD8+ TILs in malignant PCa (B) CD8+ TILs in BPH.

Serological Markers (sFas & sFasL):

Regarding to serum levels of sFas & sFasL, as shown in Table (2) the results showed the mean of sFas serum levels in patients with prostate carcinoma (3428 pg/mL ±165) is significantly higher (P value < 0.05) than those of BPH (3097 pg/mL ±270.6), whereas serum levels of sFasL had no significant difference (P value > 0.05) between BPH (409.8 pg/mL ±262) and malignant prostate adenocarcinoma (487 pg/mL ±200.9)

Table (2): Comparison of serum soluble Fas and FasL concentrations between Benign and Malignant prostate cancers.

Parameters	Benign	Malignant	P value
Fas pg/mL	3097±270.6	3428±165	0.018*
FasL pg/mL	409.8±262	487±200.9	0.523

* represents a significant difference at p<0.05. Data are expressed as Mean±SD.

Discussion

Interesting in the field of immunotherapy depending on T lymphocytes gives a hint to its importance as antitumor and protective activity against PCa cells [12]. The present study findings indicated there is no significant difference in CD3+ infiltrating cell counts between malignant and BPH. This indication consistent with Burdova et al., who mentioned that CD3+ PILs are increased in both benign and malignant PCa with no considerable difference in their counts [13]. In contrary, there is evidence that quantity of inflammatory cells comprising infiltrated T cells (CD3+ & CD8+) and B cells and macrophages are higher in prostatic benign tumors than malignant [14]. Constâncio et al., [15] and Kaur et al., [16] reported that increased CD3+ T cells and expression of androgen receptor (AR) in tumor microenvironment of prostatic malignant transformed growth are significantly associated with the poor prognosis in persons with PCa. Regarding to BPH microenvironment, there is a study showed T reg. cells quantity of infiltrated T cells were increased, and suggesting its immunosuppressive activity may leads to defeat tumor immune response [17].

Similarly, regarding to cytotoxic T cells, the current study findings suggests that tumor infiltrating CD8+ T lymphocytes are increased in both benign and malignant PCa as the same rates in agreement with Fong et al., [18] who mentioned that CD8+ PILs are increased in BPH and malignant prostate tumors. Despite its increasing

in tumor microenvironments, there are myeloid derived suppressor cells (MDSC) and T reg. cells affect the protective role of CD8+ T cells against tumor, Muthuswamy et al., [19] reported that PCa cells express high levels of MDSC and T reg attractant chemokines (CXCL-12, CCL-22) and low levels of (CCl-5 and CXCL-10) which considered as CTLs chemoattractants, and suggested celecoxib drug as CD8+ stimulator by downregulating CXCL-12 and CCL-22 and upregulating CXCL-10. Moreover, there are two reports indicated that using TGF-β resistant CTLs that produce through CAR T cells technique has specific role in killing prostatic tumor cells with prostate specific membrane antigen (PSMA) [20, 21].

In concerning to serum levels of sFas, our study results suggest the sFas levels had affected by the prostate tumor type weather benign or malignant, in agreement with Furuya et al., [22] who reported that patients with BPH had a significantly lower levels of soluble Fas than those with prostate carcinomas. Likewise, there is a study explained that increasing in concentrations mean of sFas with matrix metalloproteinase 7 (MMP 7) in prostate carcinoma had a significant role in its metastasis, by suggesting the activity of MMP 7 in metastasis process depends on concentrations of sFas [23]. There is an indication that high sFas concentrations interfere with the apoptosis to malignant transformed cells and enhance its escape from the immune surveillance [24]. Regardless to any systemic inflammatory conditions, sFas could be used for checking the response of patients to the chemotherapy or surgical tumor resection [25]. Moreover, Szarvas et al., [23] reported that patients with metastasized PCa (malignant) had no significantly difference in sFasL concentrations from those with no metastasized PCa, this may suggests that the malignant change may not affects the expression levels of sFasL, it possibly consistent with our findings that suggest there is no significant difference in serum levels of sFasL between BPH and malignant prostate adenocarcinoma. Also, it may give an indication that sFasL is constitutively released by both malignant and benign prostate tumor cells [26].

Conclusions

It was concluded there is no difference in rates of tumor infiltrating T cells and cytotoxic T cells between malignant and BPH prostate tumors. Moreover, serum levels of sFas concentrations were affected by whether malignant or benign prostate tumors, whereas sFasL concentrations were not.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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