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A comparative evaluation of tumor-infiltrating cytotoxic t cells among human urological tumors

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Abstract

Urologic malignancies are the heterogeneous neoplasms which showed as the most common in the tumors incidence rate of the world.

In this prospective cross-sectional study involved a total of (50) patients with urological tumors consist of (35) males and (15) females. The collected group of patients is distributed as the following; 23 with bladder tumor, 17 with prostate tumors, and 10 with renal tumors. Their ages ranged from (9-88) years. The biopsy was collected from each patient during the visiting of the urological surgery unit of Al-Sader Teaching Hospital-Najaf- Iraq during the period (November 2018 to October 2019).

CD8+ counts among urologic cancers (bladder, prostate, and kidney), showed a significant difference (P value = 0.05), Also, it gives an indication that the immune response in relation to intra-tumoral CD8+ T cells exhibits a disparity by the tumor site. The highest mean of CD8+ TILs counts was in bladder (101.6±69.8) followed by prostate (85.7±36.4) and kidney (49.6±34.4).

It was concluded as there is a spatial heterogeneity in counts of CD8+TILs in urological tumor sites (bladder, prostate, and kidney).

Keywords: Tumor; CD8; Bladder; Prostate; Kidney

1 Introduction

Urologic malignancies are the heterogeneous neoplasms which showed as the most common in the tumors incidence rate of the world, encompass all the tumors of genitourinary tracts: prostate, kidney, bladder, penile, and tests [1].

Urological tumors including kidney, bladder, and prostate tumors are increased with age and influenced partially by variable risk factors. Its incidence rates may increase considerably among an aging and growing population [2]. Bladder, prostate, and renal tumors are stated among the 10 most prevalent in men, in compared to that of testicular tumors which less dominant but most frequent in young [3].

Immune system dysfunction has been reported to play an important role in cancer cell invasion and metastasis as well as in fundamental biological and pathological processes, including lymphocyte homing, inflammation, hematopoiesis, and recurrent urinary tract infections. Many immunological molecules involved in the mechanism of tumorigenesis

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have been used as a marker for tumor aggressiveness in a number of malignancies such as stomach, liver, breast, and renal tumor. Understanding the role of the immune system plays in combating urological cancers continues to evolve [4]. The host immune system lymphocytes that have been seen in tumor sites known as tumor infiltrating lymphocytes (TILs), their movement to tumor sites was to withstand progression of malignant cells. Commonly, these antitumor immune cells are divided into populations and subpopulations; T cells (CD4 and CD8), natural killer cells (NKs) and non-B or non-T lymphocytes. These lymphocytes can be physically characterized by surface antigen and cluster differentiation (CD) protein [5, 6].

Immunohistochemistry (IHC) is widely recognized as the primary immunostaining technique for detecting solid tumors. In the diagnosis of urological malignancies, the immunohistochemical identification of lymphocytes may serve as a potential predictive marker [7].

The purpose of this study is to compare tumor-infiltrating cytotoxic T cells among different types of human urological tumors. By evaluating the immune response within these tumors, the study aims to provide insights into their immunological landscape.

2 Material and Methods

In this prospective cross-sectional study involved a total of (50) patients with urological tumors consist of (35) males and (15) females. The collected group of patients is distributed as the following; 23 with bladder tumor, 17 with prostate tumors, and 10 with renal tumors. Their ages ranged from (9-88) years. The biopsy was collected from each patient during the visiting of the urological surgery unit of Al-Sader Teaching Hospital-Najaf- Iraq during the period (November 2018 to October 2019).

The collected biopsies were saved in plastic tube container (10% neutral buffered formalin) to prepare for the formalin-fixed, paraffin-embedded (FFPE) technique then tissue blocks and slides for the purpose of immunohistochemical tests.

The appearance of a brown-colored reaction within the nucleus or cytoplasm was deemed indicative of a positive response. The intensity of the immunostaining was assessed using a modified approach, which involved capturing images of three distinct fields from each section with a digital camera linked to a conventional light microscope at 20X magnification. Subsequent image analysis was performed using ImageJ software (version 1.46r, National Institutes of Health, USA) for each image to quantify CD8 cells. The mean value representing the number of cells per field for each section was then calculated.

3 Results

Evaluation of the tumor-infiltrating Cytotoxic T cells among urological tumor tissues has been stated in this study. CD8+ TILs counts were studied in each urologic tumor sites (bladder, prostate, and kidney), and displayed the statistical differences among these three tumor sites, and comparison between each two sites, as shown in **Table** (1).

Table 1 Levels of Tumor-Infiltrating T Lymphocytes in Bladder, Prostate, and Kidney Cancers

Parameter	**Bladder**	**Prostate**	**Kidney**	***P value***
CD8	101.6±69.8	85.7±36.4	49.6±34.4	a- 0.05*
				b- 0.366
				c- 0.015*
				d- 0.102

* Represents a significant difference at p≤0.05. The letters (a, b, c, d) represent type of the statistical analysis: a- among the three tumor sites (bladder, prostate, kidney), b, c, d- between tumor site groups (bladder vs prostate, bladder vs kidney, and prostate vs kidney, respectively). Data are expressed as Mean±SD.

CD8+ counts among urologic cancers (bladder, prostate, and kidney), showed a significant difference (P value = 0.05), Also, it gives an indication that the immune response in relation to intra-tumoral CD8+ T cells exhibits a disparity by

the tumor site, Figure (1). The highest mean of CD8+ TILs counts was in bladder (101.6±69.8) followed by prostate (85.7±36.4) and kidney (49.6±34.4), Table (1).

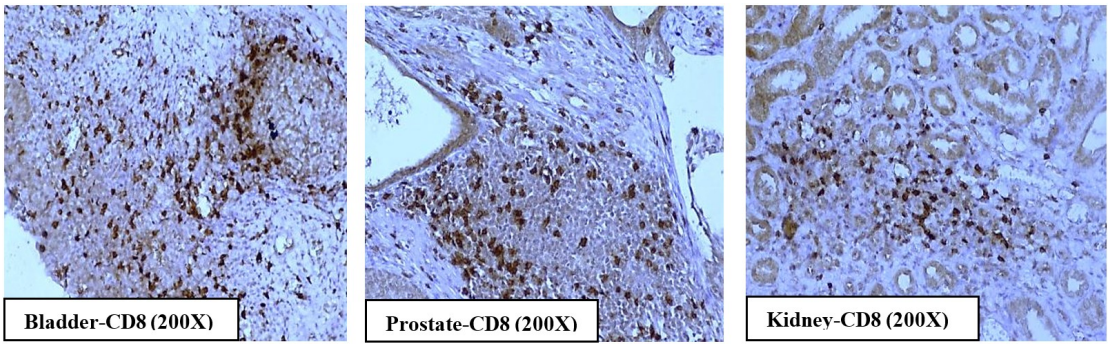

Figure 1 Exhibits a disparity of intratumoral CD8+ T cells by the tumor site (Bladder, Prostate, Kidney), the slides under light microscope, magnification power (20*10) X

The comparative analysis between bladder and prostate demonstrates that there is non-significant difference in counts of CD8+ TIL (P value > 0.05). But the quantification of CD8+ T cells in patients of bladder tumor is significantly higher than that in patients with kidney tumors (P value ˂ 0.05). Regarding to the statistical comparative analysis for CD8+ T cells between prostate and kidney tumors, the present study showed there is no significant difference (P value > 0.05) between them.

4 Discussion

The disparity of intra-tumoral CD8+ T cells also indicates that immune response can be affected by the tumor sites in agreement with Salmon et al., [8] who showed that nature of tissue and its stromal compositions affect the tumor immunogenicity. Increasing counts of CD8+ T cells in urologic tumors is very optimistic because it is considerably related with a good prognosis and outcome [9]. Moreover, in concerning to adaptive cellular immune response and anti-tumor activity, the CD8+ and Th1 cells from T lymphocyte populations are considered as the crucial in protective role against malignant cells [10, 11]. However, Nolz, [12] indicated that the function of cytotoxic T lymphocytes (CTLs) against tumor greatly depends on two important factors; (1) differentiation of CTLs; and (2) Infiltrating rate of CD8+ T cells into tumor microenvironment. Correspondingly, there is an indication that CD8αα related with the immunosuppressive role of CD8+ in reverse with CD8αβ which show high sensitivity to antigen and high stability to MHC1 binding [13, 14]. Activation of CD8+ T cells influences by IL-12 that considered as one of the most important cytokines in the enhancement of CD8+ activation in response to any immunogenic agent through induction of TCR alteration and IFN-γ production. Therefore, it is stated as one of the very important cytokines in the clinical field against tumor [15]. Moreover, among many different Toll-like receptors (TLRs), activation of TLR9 by cytosine-phosphate-guanine (CpG) dinucleotides was found to be a helpful in T cells expansion, and IFN-γ secretion which has important role in adaptive immune response through enhancement the cytotoxicity of CD8+ [16, 17].

There is a study about bladder, and another about prostate cancers support these findings by showing there is a slightly difference in CD8+ TILs count between bladder and prostate tumor tissues [18, 19]. The high density of intratumoral CTLs is very crucial in response against malignant transformation in bladder or prostate, because they are considered as the primarily responsible for elimination of tumor cells, hence a favored outcome and longer survivals [20, 21]. However, the prognostic value of general TILs depends on the tissue nature which differs from organ to others [22]. Although the strong association between CD8+ T cells and good prognosis, in prostate cancer there is a recent study indicated that PD-L1 expression is significantly associated with increased density of infiltrated CD8+ T cells, and increased risk of clinical progression [23].

The results of Laurent, [24] study about urologic cancers indicates the quantification of CD8+ T cells in patients of bladder cancer is significantly higher than that in patients with kidney cancers, and it supports finding of this study that showed the CD8+ T cells in bladder cancer tissues is significantly (P value ˂ 0.05) higher than its in kidney cancer tissues, Table (1). There is an indication revealed that increasing rates of tumor infiltrating CD8+ T cells in bladder cancer patients is considered as a great predictive for a good outcome as compared with RCC in kidney which show a poor prognosis [25, 26]. Also, using of monoclonal antibodies as immunotherapeutic agents to interrupt immune checkpoint receptors PD-1 and CTLA-4 has a notably role in upregulation of CD8 activity against malignant cells in

bladder cancer [27, 28]. Menard et al., [29] denoted that patients with RCC tumors exhibit high expansion of double positive (DP) T cells and high expression of inhibitory receptors.

Also, a text book about "advancements in cancer immunotherapy and cancer vaccines" showed that the functionality of CTLs activity against tumor was down-regulated by the same manner in both prostate and kidney cancers through CD25+T regulatory cells or by cytokines such as TGF-β that involved in their development, so the presence of CD8+ T cells in prostate and kidney cancers won't give a good outcome in compared with bladder cancer [30]. Furthermore, there is an indication that the clonal expansion of prostate infiltrating CD8+ T lymphocytes (CD8+ PIL) was done in response to unknown antigen, and undergo high expression of inhibitory receptors (PD-1 and PD-L1) which make these cells unresponsiveness against malignant transformations [31, 32]. Using of monoclonal antibodies as immunotherapy against inhibitory immune receptors (PD-1 and PD-L1) is clinically beneficial for many types of cancers, but in prostate tumors there is a resistance to these immunotherapeutic antibodies [33, 34].

5 Conclusion

It was concluded as there is a spatial heterogeneity in counts of CD8+TILs in urological tumor sites (bladder, prostate, and kidney).

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest

Statement of informed consent

The study was approved by the ethics committee, and informed consent was obtained from all subjects.

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