Comparative Analysis of Tumor Infiltrating T Cells and Serological Markers between MIBC and NMIBC Patients

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Abstract

Bladder cancer is considered as that of any type of abnormal growth arising from the tissues. It has a possibility to spread other part of the body. The study aimed to evaluate counts of tumor infiltrating immune cells (CD3 & CD8) and serum soluble Fas and Fas ligand concentrations and their comparison between MIBC and NMIBC. In this cross-sectional study a total of (23) patients with bladder cancer consist of (14) males and (9) females were included,their ages ranged from (26-82) years. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (December 2018 to October 2019). Tissue biopsy and 3 mL of blood were taken from each included subject for evaluation of CD3 and CD8 in bladder tumor tissues by IHC technique, and serum levels in sFas and sFasL in by ELISA technique. The results showed both CD3+ TILs and CD8+ TILs in MIBC are significantly higher than those of NMIBC (P value <0.0001 for CD3, and P value = 0.001 for CD8). Serum levels of sFas and sFasL in MIBC are not significantly different from those of NMIBC (P value >0.05). It was concluded that CD3+ TILs and CD8+TILs counts were significantly affected by the muscular invasiveness of bladder cancers, whereas both sFas and sFasL levels were not.

Keywords: *Bladder, MIBC, NMIBC, TILs, sFas, sFasL; Tumor Infiltrating; health.*

Introduction

Generally, the main cause of tumor incidence is genetic changes. Many years of genetic analysis showed that there are about 1000 known genes-associated cancer, categorized into three types(1) proto oncogenes that include in normal cells, but any mutation in this type of gene affecting the normal cell division causing abnormal cell growth(2) Tumor suppressor genes (TSGs) that responsible for normal cell growth, so the alteration in these genes may lead to uncontrolled cell growth, and(3) DNA repair genes, which involved in fixing and repairing in any alteration or damage in DNA, so the mutation in these type of genes leading to cancer occurrence[1].

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Bladder cancer is considered as that of any type of abnormal growth arising from the tissues. It has a possibility to spread other part of the body. Symptoms comprise hematuria, and painful urination (dysuria), and low back pain[2]. The most frequent type of CA bladder is transitional cell carcinoma (TCC); other types such as squamous cell carcinoma (SCC) and adenocarcinoma are less[3]. Most CA bladder cases are urothelial carcinomas, divided into; about 75 percent are non-muscle invasive, and 25 percent are muscle invasive[4]. Depending on the morphology, bladder tumors can be divided into papillary, solid, and mixed types. The papillary type is most frequent, particularly in non-muscle invasive bladder cancer (NMIBC), other than muscle-invasive bladder cancers (MIBC)[4].

Immunohistochemistry (IHC) is the most common application of immunostaining that used for the detection of solid tumors. Immunohistochemical detection of CD103+ tumor infiltrating lymphocytes can be used as potentially prediction factor in diagnosis of

urothelial cell carcinoma (UCC) of the bladder tissue[5]. However, there is a study validates the performance of cell detect as a urine-based assay to identify UCC in patients with history of bladder cancer. It relies on both color and morphology to differentiate between benign and malignant cells in cytology specimens[6]. Generally, tumors are closely associated with immunity system. Especially functional systemic and local immunity is required for the effective responses against tumors. In addition to an active engagement with cancer cells and tumor stroma, immune cells can be affected and are often found to be dysregulated in cancer patients[7]. Moreover, increased T cell tumor infiltration correlated with a better prognosis in most studies[8]. In cases of NMIBC, increased densities of several TIL subpopulations, including cluster of differentiation CD3+, CD4+, and CD8+ T lymphocytes, were associated with worse recurrence and survival[9].

The study aimed to evaluate the counts of tumor infiltrating immune cells (CD3 & CD8), and serum soluble Fas and Fas ligand concentrations and their comparison between MIBC and NMIBC.

Material and Method

In this cross-sectional study a total of (23) patients with bladder cancer consist of (14) males and (9) females were included. Their ages ranged from (26-82) years. Case information for each patient has been taken from the report of the diagnosis which were: name, sex, age, and the diagnosed tumor type. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (December 2018 to October 2019).

Included & Excluded Criteria: The enrollment standards of patients in this study comprised any patient who has recent bladder tumors diagnosed histologically. Patients with normal histological results after suspicion of bladder tumor, any patient who took chemotherapy, and any case with retrospective bladder tumor have been excluded.

Ethical Approval: Patients or their sons and/or first degree relatives (father & mother) were asked permission prior to take any specimen. In addition, the study concept was accepted by the Research Ethical Committee at the College of Medicine/University of Babylon.

Samples: After patient admission, the biopsies bladder tumors were obtained from the urological surgery unit. The biopsy was preserved in plastic tube container with 10 percent neutral buffered formalin (NBF), before processing for immunohistochemical testing, using paraffin embedding technique to build formalin-fixed paraffin-embedded (FFPE) blocks. Moreover, 3mL of blood were collected from each patient for serological evalution by ELISA technique.

Method

Sample Processing for the IHC Staining Technique:

- The thickness of tissue section was 4 μm taken from blocks of paraffin embedded tissue on positive charge slides when the cutting by microtome.
- Incubations specimen on chargeable slide (slide with tissue) for at least 2 hours at 58- 60 °C in the oven.
- At deparaffinization steps, 3 containers with Xylene on which the tissues/slides immersed for 2 minutes for each containers respectively.
- Then the tissues/slides dipped in 3 jars with alcohol (ethanol), the first jars with 30% alcohol, the second jars with 70% alcohol and the third jars with 100% alcohol, for 2 minutes at each jar respectively, this is hydration step.
- The last steps, subjected tissues/slides in container with Immuno DNA Retriever Citrate and then this Retrieval container with slide putin water bath set at 95 - 99°C for 60 minutes. then washed the tissues/ slides 5 time with wash buffer
- Then the immunohistochemical staining technique was applied (Bio SB- USA).

Evaluation: In IHC technique, the brown colored reaction in the nucleus or cytoplasm was considered a positive reaction. The intensity positive stained cells was determined by modified method 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]. In concerning serological markers (sFas & sFasL) were evaluated through sandwich-ELISA technique (Elabscience-USA).

Statistical Analysis: Calculation of the comparative data through Software of Statistical Package for the Social Sciences (SPSS),version 26.0,to explain the differences of study parameters between MIBC and NMIBC. Independent t-test was used for analyzing the differences. Statistically, it is considered a significant difference when P value <0.05.

Results

In the present study, there are 23 subjects of study group were diagnosed as bladder tumors. These tumors are subdivided into: 16 (69.5%) are non-muscle-invasive bladder cancers (NMIBC); and 7 (30.5%) are muscle invasive bladder cancers (MIBC). As shown in Table (1), the mean of CD3+ TILs in MIBC patients (323.5 cells/field ±19) is highly significant (P value < 0.001) higher than those of NMIBC (170±77.4). Similarly, mean of CD8+ TILs in MIBC pateints (186±61.6) is significantly (P value = 0.001) higher than those of NMIBC (64.7±29.3), Figures (1, 2). Regarding to serum soluble markers as shown in Table (2), there is no significant difference in (P value > 0.05) in means of sFas concentrations between MIBC (3251 pg/mL ±295.8) and NMIBC (3182.5 pg/mL ±351.8); also there is no significant difference (P value > 0.05)in means of Fas Lconcentrations between MIBC (474.7 pg/mL ±167) and NMIBC (374 pg/mL ±142).

Table (1): Comparison of tumor infiltrating CD3+ and CD8+ T cells counts between MIBC and NMIBC.

Parameters	MIBC	NMIBC	P value
CD3 cells/field	323.5±19	170±77.4	<0.0001*
CD8 cells/field	186±61.6	64.7±29.3	0.001*

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

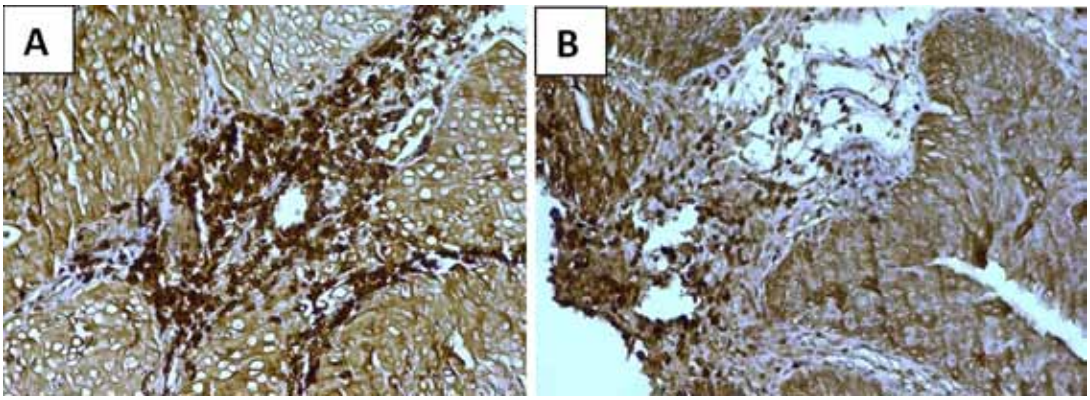

Figure (1): Bladder cancers slide with CD3 marker (20X Magnification Power): (A) CD3+ TILs in MIBCs. (B) CD3+ TILs in NMIBCs.

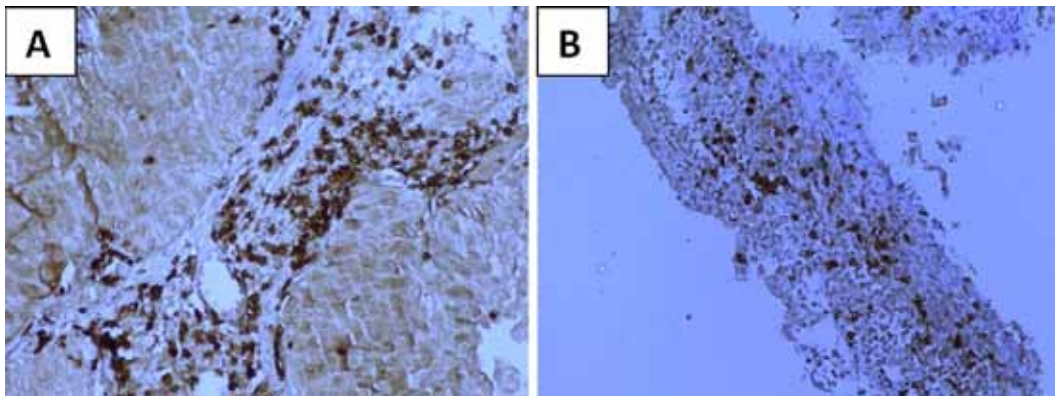

Figure (2): Bladder cancers slide with CD8 marker (20X Magnification Power): (A) CD8+ TILs in MIBCs. (B) CD8+ TILs in NMIBCs.

Table (1): Comparison of serum soluble Fas and FasL concentrations between MIBC and NMIBC.

Parameters	MIBC	NMIBC	P value
Faspg/mL	3251±295.8	3182.5±351.8	0.636
FasLpg/mL	474.7±167	374±142	0.196

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

Discussion

CD3+ TILs in bladder cancers are so important regarding to its protective role[12]. Saeed[13] mentioned that CD3+ TILs quantitation in bladder carcinoma with advanced progression stages of invasion is significantly higher than others with no muscular invasion, in agreement with the current study findings. Likewise, there is an indication was reported by Sjödahl et al.,[14], they mentioned that the general, immunological response focusing on CD3 & CD68 in bladder cancers with no muscular invasion are weaker than that with muscle invasion. Increasing ratio of T lymphocytes in MIBC may indicate to a good prognostic aspect, like the conclusion that increasing rate of tumor infiltrating T lymphocytes has a good protective consequence [15]. In contrast, there is a suggestion that the intratumoral T cells in bladder cancer are mostly accounted by T regulator cells, so it leads to suppression of immune cells of anti-tumor activity[16].

Similarly, in our study findings of increased tumor infiltrating cytotoxic T cells quantitation in MIBC tissues suggesting the rates of phenotypic T cells differs based on the degree of bladder tumor progression. The high densities of CD8+ TILs in invasive bladder cancers may indicates to a good prognosis and give a long survival rates, in agreement with that observed in invasive urothelial carcinoma, and the better prognostic aspect is associated with high CD8+ TILs quantity [17]. Also, regarding to muscle-invasive urothelial carcinoma was reported that the ratio of CTLs to T reg cells is correlated with the response to neoadjuvant chemotherapy (NAC), if the tumor infiltrating T reg cells ratio is higher than CD8+ TILs leads to unresponsiveness to NAC, and the good response was observed if the CD8+ TILs ratio is higher[18]. In NMIBC, there is no relation between CD8+ TILs counts and prognostic aspects, and observed that CD8+ infiltration is affected by the expression of fibroblast growth factor receptor 3 (FGFR3) tumor microenvironments, and high counts CD8+ TILs were observed in NMIBC with low expression of FGFR3[19]. Also, Mariathasan et al., [20] evidenced that TGF-β has a significant role to reduce the tumor infiltrating CTLs, and showed that using of anti-TGF-β antibodies facilitate the CD8+ cells diffusion into the tumor center.

Regarding to the serum soluble Fas, the current study results may indicate to that tumor invasiveness does not affect the serum levels of Fas, in agreement with Nonomura et al., [21] who articulated that sFas concentrations are not associated with tumor histological stages. But, Yang et al., [22] disagreed with our findings by showing soluble form of Fas concentrations in urine of advanced stages of urolthelial cancer are significantly higher than those with early stages, and suggest that sFas with the VEGF protein in urine may provide an improvement in treatment and diagnosis.

Despite the non-significant difference of serum levels of FasLs, the high concentrations mean of sFasL in invasive bladder cancers may be resulted from the increasing malignant cells, as evidenced that sFasL has expressed by malignant transformed cells in bladder cancers [23]. In contrary, Bahria-Sediki et al.,[24] showed the serum levels of sFasL in non-invasive bladder carcinoma are higher than that in invasive bladder cancers. This disagreement may be linked to the differences in ethnicity (Asian vs European), and measurements protocols.

Conclusion

It was concluded that CD3+ TILs and CD8+TILs counts were significantly affected by the muscular invasiveness of bladder cancers, whereas there is no differences in both sFas and sFasL levels between MIBC & NMIBC.

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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