

Correlation Between TLR-2 and Staphylococcal infection in patients with bladder diseases

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ABSTRACT

Bladder diseases are one of the most common pathological conditions in world. Cystitis is acute condition happened as a result of bacterial inflammation that can be progresses to bladder cancer. 100 urine sample were collected from patients with cystitis and bladder cancer at period from March 2021 to December 2021 in Hilla province.

This study was done to isolate staphylococcus bacteria and determine immune status for bladder diseases caused by *Staphylococcus saprophyticus* by estimating the concentration of TLR-2 as an immune marker in urine. The rate of specimens that gave a positive result for bacteriological cultures were 23.4% for bladder cancer while 76.6% negative result compare to cystitis as 70% and 30% respectively. The percentage of *S. saprophyticus* isolated and diagnosed in positive culture as a pure growth of *S. saprophyticus* in two groups, bladder cancer and cystitis was 11.8% and 14% respectively.

The incidence of cystitis and bladder cancer appear in both sexes, male showed highly rate of infection than female 68% , 32.6% respectively . Bladder diseases were appeared in different age group, (56-71) years showed highly infection rate. Immunological test to urine of patients with bladder diseases referred to significant increasing in the concentration of TLR-2 for all patients with bladder cancer at different age groups, compared to cystitis and highest concentration of them in the age group (56-71) years (7.696 ± 0.235 ng/ml) , This study referred to the role of cellular immune response through bladder diseases.

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INTRODUCTION

The urinary bladder is a hollow organ located in the lower abdomen that stores urine from the kidneys (through the ureter) until urination. Urothelial cells, which line the urinary¹ bladder¹ and urinary tract, are specialized transitional epithelial cells that accommodate the volume. Smooth muscle lines the bladder, which may relax to accept larger quantities and contract¹ (under voluntary or reflex control)¹ to expel urine down the urethra¹ and out of the body (Zhuo et al, 2021).

One of the most prevalent urinary tract illnesses is cystitis. The word cystitis was developed to describe the infection's location, which termed to bladder infection, which is usually caused by bacteria from intestinal flora (Ki et al, 2017; Huether, 2019) *S.saprophyticus* was discovered to be the second most prevalent cause of cystitis, behind *E. coli*. It has unique urotropic and ecological characteristics that distinguish it from other staphylococci and *Escherichia coli* (Dolores et al, 2020).

The development of tumors and their transformation into malignancies is occasionally under the influence of a group of microorganisms. Bacteria, which are one of the major causes, accounting for more than 20% of human malignant tumors (Garrett et al, 2015; Urbaniak et al,2016). Bladder cancer (BC) is a type of solid tumor that is represent a complex mass of cells that interfere with each other to stimulate a wide range of immunological markers that play a role in the diagnosis of Microenvironment (Petitperz et al, 2020).

If uropathogens breach the physical barriers of bladder epithelium, they are recognized by TLR subfamilies group such as TLR2 recognizes staphylococci by their lipoteichoic acid or lipoprotein (Dutta et al, 2020). Toll-like receptors An immune surveillance molecules family, TLRs are set of germline-encoded receptors , which are called pattern recognition receptors (PRRs) as proper function of innate immunity dependent ,that bind pathogen-associated molecular patterns (PAMP) and are an important

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part of the innate immune system’s defense against invaders (Timur et al, 2020). Gram positive bacterial component (ligand) activates TLR2 to produce inflammatory responses. Only lipoproteins/ lipopeptides (LPs) are “true” TLR2 ligands, according to Hoppstädter et al. research, since they are detected at physiological quantities by this receptor (Hoppstadter et al, 2019). Immune cells, endothelial cells, and epithelial cells have all been found to express TLR2 . This might indicate an early bladder immune response prior to the onset of symptomatic UTIs, supporting TLR2’s potential protective function against UTIs (Livia and Molly, 2021).

MATERIALS AND METHODS

The study included 100 patients with different ages suffering from cystitis and bladder cancer at period from March 2021 to December 2021 in Hilla provinc , after recording information about patients.

Collection 3ml of urine from all patients with bladder disease in clean dry tube, then put in centrifuge at 2500-300 rpm, supernatant stored for immunological test and take sedimentation for bacterial culture.

Each sample separately were cultured on (Nutrient agar, UTI Chrom agar and Manitol salt agar) under sterile conditions and specimens were cultured for 18-24 hours at 37° C after incubation, bacterial growth of most samples was investigated visually and microscopically using VITEK method (Fawole and Oso,1998).

Immunological test: The study contributed also measuring the concentration of TLR-2 of cystitis and bladder cancer patients in urine by ELISA technique according to the manufacturer’s instructions.

Statistical Analysis

To do statistical analysis, use the U.S. Census (spss 11), as well as the design random full-scale analysis of variance and the test for less significant differences. Below the 0.05 threshold of significance, the least significant difference test (LSD) and table analysis of variance (ANOVA Table) are used (Niazi,2004).

RESULTS:

In the this study, it was found that the highest percentage of infection was recorded in males by 68% and 32% for female (Fig.1).

The infection was divided between the different age groups, which ranged from [40-87] years. The highest percentage of infection was found in the age group [56-71] years. It was the highest rate of 50%, followed by the age group [40-55] the lowest incidence rate in the age group [72-87] was 20% (Fig.2).

The current study showed that the urine samples that gave a positive result for bacteriological cultures were 23.4% for bladder cancer while 76.6% negative result compare to cystitis sample as 70% and 30% respectively (Fig.3).

This study also examined the isolation and diagnosis of gram positive bacteria and showed only *Staphylococcus saprophyticus* as pure culture in two groups bladder cancer and cystitis. By 11.8% and 14% respectively (Fig. 4).

The current study’s findings indicated that there was a significant rise (P0.05) in the concentration of TLR-2 and for all age groups of patients with bladder cancer compared to cystitis its subsidiaries¹ through the use of technology¹ calibration absorbance linked immunoassay Enzyme - Linked Immunabsorbent Assay (ELISA), and the highest levels appeared at the age group of 56-71year (table 1).

DISCUSSION

This study showed that people who suffered from bladder diseases were more susceptible to bacterial infection especially *Staphylococcus saprophyticus*. The present study demonstrated that bladder cancer and cystitis was distributed in both human

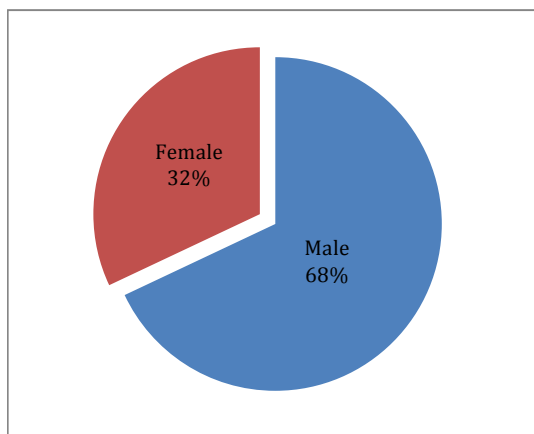


Figure 1: Distribution of infection according to the sex

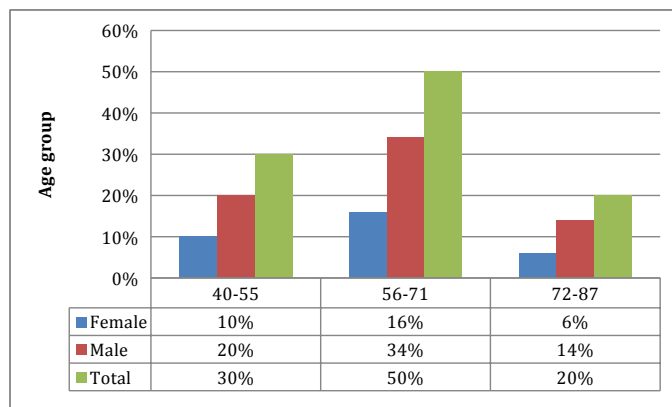


Fig. 2: Distribution of infection according to the age group

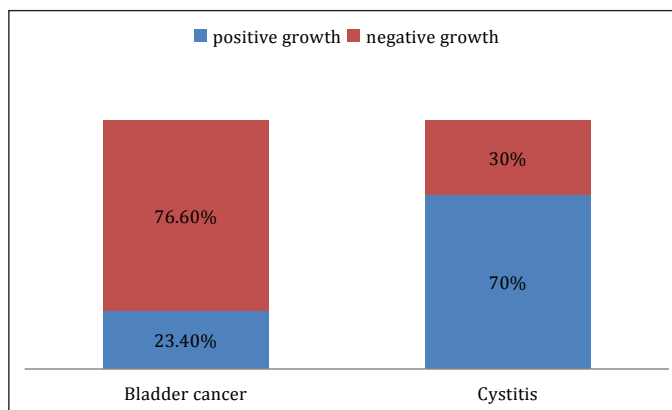


Fig. 3: Percentage of bacterial growth for urine culture with bladder diseases

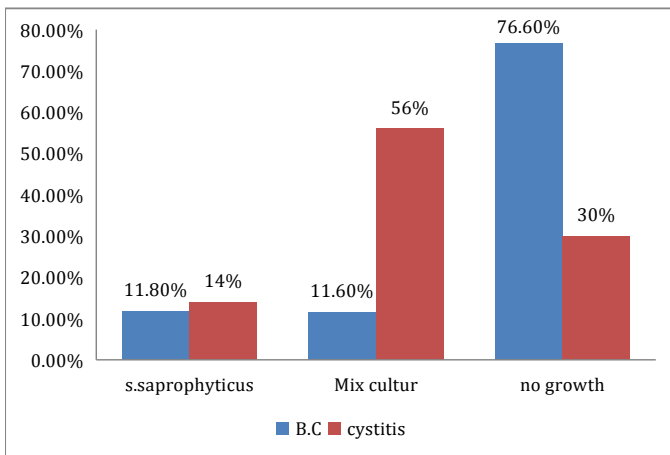


Fig. 4: Percentage of *S. saprophyticus* isolation in bladder diseases

sexes with highest percentage was recorded in males by 68% compared to other studies, Current results were consistent with other studies (Siegel et al, 2019), indicating that bladder diseases are more prevalent in the age group more than 55 years about 80-90% and prevalence among females than males depending on many factors. Personal history increases the risk of bladder cancer and is mainly associated with early onset (Kalyan et al, 2020).

Study showed the bacteriological examination for patients urine supported the diagnosis and explain inflammation state in bladder. The positive result of the bacterial culture is critical in determining the type of antibiotic to use for treatment, though it does not always stipulate a valid diagnosis, as many studies have shown that the results of the transplant interfere with the normal flora. Otherwise, is linked to variables that raise the oscillation of infection or failure to respond to antibiotic treatment (Karamali et al, 2019). The surge in negative bladder cancer outcomes might imply that many of the medicines and antibiotics used to strengthen patients' immune systems are working. Furthermore, in cases of cystitis, the non-bacterial infection is caused by a viral or fungal infection (Lene et al, 2020).

A bacterial infection of the urinary bladder is the most common cause of acute cystitis. Prevalence of *Staphylococcus saprophyticus* in study as pure culture reached to 11.8% with bladder cancer compared with cystitis group about 14%. Results were consistent with recent study (Lawal et al, 2021) discovered that *S. Saprophyticus* producing UTI in humans belonged to two primary clonal lineages (G and S), which arose in food/production animals and humans, respectively (Noor et al, 2013; Karla et al, 2020). And it has ability to inhibit other bacteria's development via generating the urease enzyme, haemagglutination, and adhesion to human uroepithelial cells, are among the virulence characteristics (Agwal et al, 2020).

Although the immune response in some diseases especially cancer has been well characterized (Ayala et al, 2019), the mechanisms involved in reactivation in humans are still unclear. Knowledge of the anti-tumor and anti-inflammation immune response is important. Present studies pointed that the concentration of TLR-2 in urine is a result of systemic overproduction because of dendritic cells, neutrophils and regulatory T cells (T regs) activation by bacteria contacts

Table 1: Level of TLR-2 in patients with bladder diseases

Age group	Groups	Concentration of TLR-2 ng/mL Mean \pm S.D
40-55 y	Cystitis	4.352 \pm 0.245
	Bladder cancer	5.55 \pm 0.284*
56-71 y	Cystitis	2.577 \pm 0.088
	Bladder cancer	7.696 \pm 0.235*
72-87 y	Cystitis	1.374 \pm 0.104
	Bladder cancer	2.675 \pm 0.161*

*L.S.D under (P<0.05) = 0.362

with TLR-2 receptor, which plays an essential function as a biomarker in the innate immune response, according to (Joseph et al, 2019). The present study revealed a significant increasing in the concentration of TLR-2 for all age groups of patients with bladder cancer compared to cystitis and the highest concentration of them for the age group 56-71 years. Study evidence pointing to an important immunological role of TLR-2 in controlling infections caused by Gram-positive bacteria in general, but it is possible that another group of receptors may be involved in controlling Gr+ve bacteria when they are not secreted or activated (Ohadian et al, 2019). Toll-Like Receptors activation in tumor cells may have both tumorigenic (positive regulation of cancer) and antitumor (negative regulation of cancer) effects (Hoppstadter et al, 2019).

The immune response accompanying tumors includes both humeral and cellular types in an integrated manner, and therefore it is noted that both innate¹ and adaptive¹ immunity are involved to limit tumor development. Immune cells can be harmed by cancer and are frequently shown to be dysregulated in patients (Patras et al, 2019).

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