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ORIGINAL ARTICLE

Bacteriuria in Patients with Orthotopic Ileal Neobladder: The Role of Uropathogenic *Escherichia coli*

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ABSTRACT

Urinary diversion is mainly needed after radical cystectomy. Urinary tract infection is one of the most common infectious complication following this procedure and *Escherichia coli* is the most prevalent organism implicated in bacteriuria associated with orthotopic ileal neobladder.

Abbreviations:
ASB: asymptomatic bacteriuria**, BC:** bladder cancer, ***E. coli***: *Escherichia coli*, **GIT:** gastrointestinal tract, **MLST:** multi-locus sequence typing, **PAI(s):** pathogenicity islands, **PFGE:** pulsed-field gel electrophoresis**, RC:** radical cystectomy, **UD:** urinary diversion, **UPEC:** uropathogenic *Escherichia coli*, **UT:** urinary tract, **UTI(s):** urinary tract infection(s), **VF(s):** virulence factor(s).

INTRODUCTION

Urinary diversion (UD) is principally indicated for managing bladder cancer (BC) as radical cystectomy (RC) is considered the main treatment modality for this cancer type. Additionally, UD can be needed in other conditions such as neurological disorders, congenital malformations, trauma, and some forms of cystitis. A transposed gut segment is manipulated to divert urine flow after the diseased bladder is being excised. To develop a bladder substitute after excision is a challenging issue[1].

A study from the Urology and Nephrology Center, Mansoura University, Egypt, stated that between the year 1970 and 2000, about 2090 men and 630 women with invasive BC were treated with RC and UD[2].

In orthotopic UD, the excised bladder could be substituted using a number of different surgical maneuvers and with different tissues types, the most popular is the reconstruction of an orthotopic ileal neobladder which has gained wide acceptance as the favorable method of UD after RC especially in young patients as it offers better quality of life, and superior body image with similar net results to ileal conduit diversion[1,3]

However, RC with UD is a morbid and complex maneuver with high rate of postoperative complications ranging from 49% to 78%. Basically, infectious complications, with urinary tract infections (UTIs) in specific, are the commonest with clinical manifestations including asymptomatic bacteriuria (ASB), symptomatic UTI, or less frequently, urosepsis[4]

Escherichia coli (*E. coli*) is considered as the most common involved bacterial agent causing neobladder-related UTI which was found to be responsible for about 60% of monobacterial infections. An important point to consider is that *E. coli* strains existed for lengthy periods in spite of the various antimicrobial regimens followed[5].

A conflicting evidence presents as regard the pathogenic potential of *E. coli* strains colonizing the reconstructed neobladder. It was demonstrated that these strains isolated from neobladder are more similar to those isolated from patients suffering from asymptomatic or uncomplicated UTI using multi-locus sequence typing (MLST) and directed PCR[6]. On the other hand, neobladder strains were found to have a higher pathogenic potential compared to fecal isolates when focusing examination on uropathogenic virulence factors (VFs) using pulsed-field gel electrophoresis (PFGE)[5].

Urinary diversion: Orthotopic ileal neobladder is the favorable

There are three main procedures for replacing the excised bladder. The non-continent UD is the first, where the ureters are connected to one end of a separated intestinal loop and the other end is manipulated to create a stoma on the anterior abdominal wall (Fig.1a). The second is the continent non-orthotopic diversion which involves construction of a

continence mechanism that needs periodic self-catheterization of the newly developed intestinal pouch (Fig.1b). The third one is the orthotopic UD, where a neobladder is created from an intestinal loop and is then connected to the urethra (Fig.1c)[7].

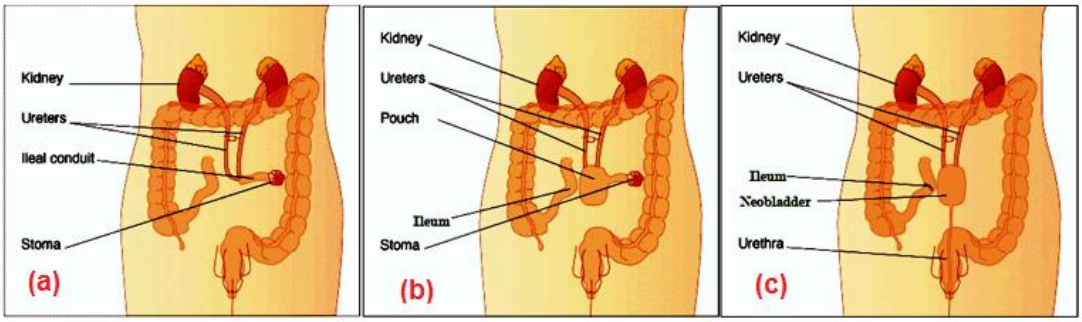

Fig. 1: The Three mainsurgical procedures of replacing the excised bladder. (a) Noncontinent UD, (b) Continent UD, (c) Orthotopic neobladder[3].

As a result of its influence on urinary control, body image, and sexual function, RC plus incontinent external UD is considered as one of the most traumatic cancer surgeries as regard physical and emotional burden and lifestyle alteration. These drawbacks have improved with the development of orthotopic bladder replacement, which is essentially considered as the favorable procedure for continent UD[1].

All parts of the gastrointestinal tract (GIT) have been evaluated for reconstruction of an orthotopic neobladder, but comparing all different segments used showed superiority of the ileum over the others[8]. Many forms for remodeling an orthotopic ileal neobladder have been depicted using small intestinal loops reconfigured into several forms such as U, crossfolded U, W, M, V, in a spherical manner and with different strategies for the connecting the newly developed reservoir to the ureters. The most popular reconfigurations are the Studer and Hautmann ileal neobladder[9].

Orthotopic ileal neobladder-associated bacteriuria: Urinary tract infection or asymptomatic bacteriuria?

While orthotopic ileal neobladder provides the patients with excellent functional results with better quality of life, this actually comes on the expense of elevated rates of UTI[10].

It is estimated that about 50% of the patients suffered at least one attack of symptomatic UTI within their first year of postoperative follow-up. It usually occurred within the first three months post surgically then plateauing at one year afterwards[11].

Despite the prevalent bacterial colonization that occurs in the reconstructed neobladder, manifestations of local infection are not common. Symptomatic infection of the orthotopic neobladder that is usually called pouchitis, has been reported, but is not abundant. On the other hand, clinical reports reinforce the concept that bacteriuria associated with neobbladder in most of the cases is similar to the ASB state in individuals with native bladder[12].

A main difference is that, ASB in the native urinary tract (UT) involves carrying a single strain, usually *E. coli* in the majority of cases, for a prolonged periods. On the contrary, in patients with neobladder, bacteriuria is distinguished by the frequent presence of multiple strains, from which only some strains exist for lengthy periods[13]. The etiology of the detectable lack of the host immune stimulation may be explained by intrinsic properties the mucosa of the transposed intestinal segment[5].

The diagnosis of UTI in such patients with UD is challenging due to its vague clinical presentation and the lack of a consistent definition. Clinical presentation vary from being asymptomatic up to sepsis; common symptoms may include dysuria, flank lower or abdominal pain, fever, chills, and alterations in the urine's gross criteria[11].

While ASB is frequent in orthotopic neobladder affecting 30% to 80% of patients post surgically, reported rates of symptomatic UTI are considerably lower[14]. A greater risk of neobladder colonization could be associated with a variety of risk factors including female gender, incomplete emptying of the neobladder, high body mass index, and the presence comorbidities such as diabetes mellitus[11].

The factors involved in the development of bacterial colonization of neobladders may include impaired emptying, excessive mucus secretion, lack of some of the natural defenses against bacterial adherence, and the inherited immune tolerance of the transposed gut loop, all together result in the loss of the mechanical and immunological defenses existing in native bladders.

This high colonization rate eventually may cause a higher rate of symptomatic UTI[3,18].

Functional and histologic changes associated with the maturation of the orthotropic neobladder have been suggested to play a central role in lowering the rate of UTI noticed in these patients over time. There is a marked increase in maximal flow rate associated with increased neobladder capacity resulting in the decreased residual urine volume that is noticed over a 6-month interval post surgical, finally leading to the decreased rate of UTI episodes[15, 17].

Patients with maximal neobladder emptying have a much lower rate of bacteriuria. Consequently, it is considered that regular and complete emptying represents a vital defense mechanism against UTI[16].

Treating ileal neobladder-associated ASB is conflicting. Some authors recommend prophylactic antibiotics in those patients with recurrent UTI, however the majority do not regard it mandatory to treat positive urine cultures despite the lack of specific urinary symptoms[16].

Antibiotic suitability testing showed that isolates were mostly susceptible to amikacin. Thus, amikacin is initially recommended as empirical treatment for those with symptomatic UTI associated with UD taking into consideration the patient's renal function[19].

The battle with the body defenses: The roles have changed

Specific and native defenses

The native defenses have a wide spectrum and include epithelial defensins found on all mucosal surfaces, mechanical forces such as urine flow and regular emptying of the bladder, mucin production in the GIT, inflammation, etc[20].

The local defenses against bacterial invasion are compromised or even lost upon the reconstruction of the lower UT. Nevertheless, the intrinsic properties of the transposed intestinal loop carry complex native and specific defenses against infection beside the existing mucosal antigenic tolerance mechanisms[21].

Innate antibacterial defenses in the reconstructed bladder

Inflammation

The normal UT is sterile, as a result of the presence of potent anti-bacterial defense. It was found that mucosal inflammation is essential for the early clearance of bacterial cell from the intact UT[20].

Pyuria was found to present in most neobladder urine samples, as well as in sterile urine. Additionally, urine from colonic neobladder patients was found to have absent or low levels IL-6 and IL-8 compared the markedly higher levels that present in urine from ileal neobladders. Hence, it could be concluded that ASB in in case of neobladders results in a substantial but still limited local mucosal response that is thought to be higher in ileal neobladder patients[12].

It was found that *E. coli* isolates recovered from patients with ASB after neobladder reconstruction carried less frequent virulence traits compared to UPEC recovered from patient with community-acquired UTI. In order to successfully colonize the reconstructed neobladder, *E. coli* seems not to be relying on usually identified virulence traits, supporting the concept that colonization is asymptomatic, and usually associated with a limited host response[5].

Mucus

Adherence to the mucosal surface is vital for virulence. In *E. coli* it is mainly mediated via pili that present on the surface of the bacterial cell. However, the mucosa of the intestine possesses mechanisms which can block the attachment of the microbes to the enterocytes[25].

The intestinal mucus has specific receptors to which *E. coli* pilus adhesins can attach, these receptors are similar to those of the enterocytes. The attachment of *E. coli* to the enterocytes was found to be blocked by goblet cell-derived mucin. [26] Entrapment of bacteria to mucus could block their adhesion to enterocyte, possibly via competitive inhibition, despite non-specific interactions may also present. Thus, in reconstructed UT, the mucus may act as an effective host defense[12].

Residual urine

Even in the most perfect case, neobladder emptying results in formation of intestinal folds that may act as small pouches of residual urine, usually in volumes enough to maintain a state of stable bacteriuria. As in the normal UT, neobladder patients with very small volumes of residual urine (<20 ml) have a marked lower rate of bacteriuria, or even sterile urine compared to those with larger[13].

Type of interposed intestine

Mixed flora was found to be more abundant with ileal than with colonic conduits, and monobacterial cultures were prevalent in case of colonic continent reservoirs in contrast to ileal neobladder group which always had bacteriuria even with maximum emptying (residual urine <20 ml)**,** while colonic neobladders were found to have sterile urine[12].

Urine cultures from colonic neobladder, usually show prevalence of non-pathogenic aerobic strains, while in ileal neobladder anaerobic bacteria were markedly dominated. Further, *Ureoplasma urealyticum* was detected only in urine cultures from patient with colonic neobladder. Also, the colonic tissue was found to be more resistant against *E. coli* infection[13]. These data postulate that colonic and ileal tissues provide different ecological niches that may differently affect the local response[27].

The role the gastrointestinal immune responses in the reconstructed neobladder

The intestinal mucosa is equipped with several tools, that constitute the intestinal mucosal barrier to face the

bacterial colonization and other antigens found in the GIT[22].

The locally produced immunoglobulins continue to be formed in the excised intestinal loops incorporated into the reconstructed UT and it is suggested to retain its local properties. For example, a response to antigens in the neobladder, is associated with secretion of IgA in the interposed loop but not in any other place in the gut[23].

The elevated levels of s-IgA in neobladder urine were noticed to be more efficient in blocking bacterial adherence to urothelial cells than s-IgA found in native bladder urine[12].

It was found that s-IgA could to be trapped within the mucus gel and become concentrated near the mucosal surface as a result of the strong non-covalent interaction between mucus and s-IgA. Thus, providing a specific immune sheltering[24].

Uropathogenic *E. coli* and the pathogenesis of bacteriuria in urinary diversion

Uropathogenic *E. coli* (UPEC) strains are equipped with a variety of VFs closely associated with colonization, persistence, and pathogenesis of bacteria in the UT. The most vital of these factors include biofilm formation, adhesins or fimbriae, and toxins such as hemolysin[28].

Urovirulence factors of uropathogenic *E. coli*

As *E. coli* is considered the most incriminated cause of UTI, many studies have suggested that there are special properties or VFs that allow the bacteria to colonize the UT and persist against the highly effective host defense strategies including the urine flow, exfoliation of urothelial cells, invading neutrophils, and endogenous antimicrobial factors. As mentioned, UPEC strains show a high degree of genetic diversity as result of having specified virulence genes that present on mobile genetic elements called pathogenicity islands (PAIs)[29, 36].

In fact, there is no certain factor that enable the differentiation of UPEC from the commensal fecal flora except for the ability to enter the UT and cause infection[30].

However, *E. coli* can be an infectious agent in the UT that causes disease by the cumulative impact of one or several VFs Figure (). Investigation of the VFs in certain strains is very important to help understand the mechanism of action of the virulence properties and also to develop specific anti-virulence interventions to prevent infection[31].

At the basic level of epidemiology, VFs of UPEC can be categorized by comparing the host population variation (e.g. male vs female, pregnant vs non-pregnant women, patient with normal vs abnormal or instrumented UT). This comparison reflected the correlation between a particular VF with the host-parasite interactions[32].

Also, potential findings of VFs from UPEC can be identified by comparing the properties of the bacteria, which have been isolated as a main cause of UTI, with fecal strains from healthy subjects[33].

In addition, the severity of the clinical syndrome of UTI is an important factor in categorizing urinary isolates for the purpose of identifying more potential VFs. Suspected VFs have also been identified using localization methods to determine the upper (kidney and ureter) and lower (bladder and urethra) uropathogenic isolates[28].

However, a true prevalence of VFs can be obtained by combined results from a variety of epidemiological studies. In addition, identification and understanding the VFs of UPEC and their mechanism of action facilitate practical applications for more precise approaches in phenotypic or molecular diagnosis and epidemiology[34].

It was demonstrated that VFs of UPEC that have been theoretically implicated in the pathogenesis of UTIs can be divided into the following categories as shown in figure (2): those associated with the bacterial cell surface, those which are secreted and exported to the site of action (toxins), iorn-acquisition systems and immune evasion mechanisms[35].

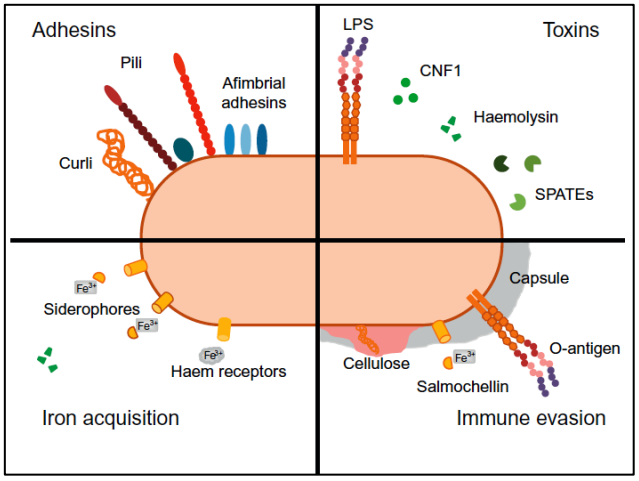

Fig. 2: Fitness and virulence factors of uropathogenic *E. coli*[30].

It was documented that PAIs associated with the genome of pathogenic strains retain coordinate horizontal transfer of virulence genes between strains of one species or related species[37].

The mostly accepted theory is that UPEC arose from non-pathogenic strains through gaining new VFs by horizontal DNA transfer of these PAIs that present on chromosomal or plasmid level[38].

The majority of the pathogenic *E. coli* strains are not thought have a single evolutionary origin or monophyletic group, as they arise from several events of gene transfer**.** Using a triplex PCR, phylogenetic

analysis is based on the presence or absence of three gene markers including chuA (an outer membrane hemin receptor gene), yja the gene encoding for an uncharacterized protein (a gene encoding for an uncharacterized protein) and TSPE4.C2 (an unspecified DNA fragment). Consequently, various *E. coli* strains could be classified into 4 major phylogenetic groups including A, B1, B2, and D. These phylogroups can be subdivided into 7 subgroups A0, A1, B1, B2(2), B2(3), D1 and, D2 using a rapid PCR[39].

It was found that isolates belonging to group B2 and D are commonly more virulent, while the majority of group A and B1 isolates are nonpathogenic and isolated mainly from gut microbiota[40].

CONCLUSION

Bacteriuria is one of the most frequent post-operative events after RC with UD. It is promoted by the transposition of intestinal segments into the UT. In most of the cases the bacterial carriage is asymptomatic, and usually associated with absence of a systemic host immune response. *E. coli* is among the most common implicated bacterial agent and usually persist for long periods despite the use of antibiotics, and is mostly sensitive to amikacin.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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