Original Article

Isolation and Identification of Bacteria Associated with Bladder Cancer Patients

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Summary:

Background: Several biological factors such as bacterial infections and immunological status are implicated in predisposing individuals to bladder cancer. Bacterial infection of urinary tract has been related to increase the risk of bladder cancer.

Patients and Methods: Resected tumors of a total of 73 patients were obtained under sterile surgical conditions. Biopsy processing samples and culture procedures of biopsy samples were mentioned in the text.

Results: Bacterial growth was observed in 48 biopsy tissues of those patients represent (65.8%) while, 25(34.2%) yielded no growth (negative results).

It is obvious that *E. coli* is the most predominant organisms followed by *K. pneumoniae* and *Ps. Aeruginosa*. The other uropathogens isolated more or less of equal distribution.

Conclusion: High frequency and great variety of bacteria in cystectomy specimens removed from cancer of urinary bladder. They were often Gram-negative pathogens (primarily Enterobacteriaceae).

Key words: Bladder cancer. Bacterial infection**.**

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Introduction:

Several biological factors such as bacterial infections and immunological status are implicated in predisposing individuals to bladder cance (1, 2). Bladder cancer (BC) carcinogenesis is probably related to bacterial and viral infections, commonly associated with bilharzial infection rather than the parasite itself (3).

Bacterial infection of urinary tract has been reported to increase the risk of BC (4). Significant etiological factors have not been identified, but chronic inflammation caused by infectious agent appears to playing a role in this disease (5).

Bladder cancer in particular Sequamous Cell Carcinoma (SCC) also may be caused by chronic cystitis (bladder inflammation) due to long term urinary tract infection (UTI)(6). Chronic urinary tract infections are thought to contribute to bladder carcinogenesis by several mechanisms. Repeated chronic irritation can lead to metaplastic changes, then dysplasia, and finally carcinoma. Glandular metaplasia may be seen in cases of calculus, chronic bacterial infections particularly due to *Escherichia coli*, schistosomiasis and in extrophy of the bladder (7).

The best-documented relationship between bacterial infection and malignancy is *Helicobacter pylori* and gastric carcinoma. In early childhood, *H. pylori* alter the gastric mucosa at the cellular level resulting in chronic inflammation. This inflammation is in turn thought to cause cancer by inducing cell proliferation(8).A recent study of El-Omar *et al.* (2000)[9] shows that the pro-inflammatory genotypes of the interleukin-1 loci increases the probability of the establishment of a chronic hypochlorhydric condition in the corpus of the stomach. This condition favors the development of bacterial infections and thus the production of reactive oxygen and nitrogen oxide species that are mutagenic and carcinogenic. Recently, however, bacteria have been linked to cancer by induction of chronic antigen exposure or production of carcinogenic metabolites (10). The relation between infection and tumors is not limited to the stomach only, Alberchet *et al.*[11], observed that certain microorganisms such as *Borelia* can cause lymphoma cutis.

Mycoplasma-like organisms have been suggested to be associated with Hodgkin's disease (12). Recently, an observation that suggests an association between Chlamydia pneumoniae and cutaneous T-cell lymphoma has been published (13).

The present investigation is a trial to isolate bacteria from resected bladder tumors.

Material & Methods:

A total of seventy-three Iraqi patient with bladder tumor (53 males, 20 females) were investigated. The mean age of patients was (60) year (range 29 to 83). The patients attending the specialized Surgical Hospital in the Medical City Teaching Hospital in Baghdad from different areas of Iraq. Tissue biopsy material of the bladder tumor obtained from operation

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was placed into the sterile container with wide mouthed screw capped bottle was used for bacteriological examination. Small amounts of sterile, non-bacteriostatic saline were added to keep the specimens moist. Biopsies were minced and emulsion was prepared in a mortar in sterile conditions. A loop full of the emulsion transferred to glucose broth or brain heart infusion broth and these cultures incubated for several days until the turbidity appear. The loop full from the turbidity was transferred to new culture medium by streaking on blood and MacConkey agar plates. The plates were incubated aerobically at 37°C for 24 hours then examined for bacterial growth. The bacterial isolates were identified by using different systems such as API 20E and API STAPH were carried out to validate bacterial species (14).

Results:

An attempt to isolate pathogens associated with bladder cancer tumors, particularly bacterial pathogens was done. Resected tumors of a total of 73 patients were obtained under sterile surgical conditions.

Bacterial growth was observed in 48 biopsy tissues of those patients represent (65.8%) while, 25(34.2%) yielded no growth (negative results).

Table (1) illustrates the isolation and identification of bacteria from positive culture biopsy of 48 patients with urinary bladder cancer.

Total No. of patients	Type of culture isolates	No.	%	Bacterial isolates	No.	%
48	Pure bacterial growth	42	87.5	Escherichia coli	14	33.3
				Klebsiella pneumoniae	11	26.2
				Klebsiella oxytoca	3	7.1
				Pseudomonas aeruginosa	8	19.0
				Proteus mirabilis	3	7.1
				Staphylococcus aureus	2	4.8
				Staphylococcus faecalis	1	2.4
	Mixed bacterial growth	6	12.5	Escherichia coli & Pseudomonas aeruginosa	3	50
				Escherichia coli & Staphylococcus aureus	1	16.7
				Klebsiella pneumoniae & Pseudomonas aeruginosa	1	16.7
				Klebsiella pneumoniae & Staphylococcus aureus	1	16.7

From the above results, it is obvious that *E. coli* is the most predominant organisms followed by *K. pneumoniae* and *Ps. aeruginosa*. The other uropathogens isolated more or less of equal distribution.

Discussion:

Appell *et al.* (1980)(15) demonstrates that the source of bacteriuria after transurethral resection of tumor is the tumor itself, he showed that the bacteria are not introduced during cystoscopy but they are present within the neoplasm and are merely release during resection of bladder tumor. However, Lattimer and Tannenbaum (1997)(16) observed at high frequency and great variety of bacteria in urethrectomy specimens removed for cancer urethra. They were often Gram-negative pathogens (primarily Enterobacteriaceae). Bacterial infection has not traditionally been considered as a major causes of cancer (5). Several epidemiological studies have suggested that infection or inflammation of the urinary tract may be a risk factor for cancer of bladder (17). When phagocytes (neurophils, eosinophils, monocytes, macrophages) are exposed to an inflammatory stimulus (e.g. bacteria), they become activated and begin to generate large quantities of reactive oxygen and nitrogen intermediates that could lead to direct DNA damage (18). Reactive oxygen intermediates, also generically referred to as oxidants, are derivatives of molecular oxygen such as superoxide, hydrogen peroxide, hypochlorous acid, singlet oxygen, and the hydroxyl radical. Under normal circumstances, phagocyte-derived oxidants serve a protective function by killing invading bacteria and parasites. However, they can also have detrimental effects causing tissue damage and contributing to the development or progression of numerous diseases including cancer(18). Furthermore, *E. coli* isolates that seems to be the major bacterial species was found in biopsy culture in 14(33.3%) as a single isolates. This finding is in agreement with the study performed by Hassani (2003)[19] in Iraq who reported that *E. coli* isolates from biopsy tissues of posterior urethral tumors represented predominant bacterial species found in eight patients out of 15 patients represent (20%)

In the present study, we observed these isolates which were found in mixed culture with other bacteria like *Ps. aeruginosa* and *S. auerus* represent 50% and 16.7% respectively. In addition, these findings were confirmed by Hassani (2003)[19] who found that *E. coli* was observed in mixed culture with *P. mirablis* represented (62.5%). *K. peumoniae* and *Ps. Aeruginosa* 25% then, *Enterobacter aerogens* 12.5%.

Furthermore, *E. coli* and *Ps. aeruginosa* have specific virulence factors that allow to survive, replicate and stimulate cell invasiveness.

E. coli is the most common organism causing UTIs and predominates strongly at most ages (20). In

additional to virulence factors like O-antigen, K-antigens, haemolysin production, haemagglutination of human RBCs, adhesions, aerobactin and colicin production then resistance to bactericidal effect of human serum.

On the other hand, *K. pneumoniae* observed in 11(26.2%) of the cases as a single isolate and mixed isolate only in two cases with *Ps. aeruginosa* represent (16.7%) and *S. aureus* (16.7%). The incidence of this bacteria may be related to virulence properties. It was contributed that this bacterium can adhere to the epithelial cells and colonize the infected tissue and may be ascribe to their opportunistic ability particularly when there was some lowered tissue resistance or other predisposing factors like production of cytotoxins(21,22) enterotoxins(23)and hemolysin(24). *Ps. Aeruginosa* is another bacterial isolate found in biopsy culture of the same patients present as a single infection or dual infections with *E. coli* (50%) or *S. aureus* (16.7%). Most of the isolated strains from clinical specimens produce large number of virulence-associated exoproducts. These include exoenzyme S, the protolytic enzymes alkaline proteases, elastases, collagenases, two hemolysins: a heat-labile phosphatase C and heat-stable glycolipid, and exotoxin, which is comparable in action to diphtherial toxin(20).

Notably, existence of mixed infections in tissue cultures may support bacterial ability to cause progressive lesion(25). Whereas, the existence of *S. aureus* in biopsy tumors patient may be related to their ability to multiply and spread widely in tissues through their production many extracellular substance. Some of these include enzymes such as catalase, coagulase, hyaluronidase, staphylokinase, while others are considered to be toxins such as haemolysin(26). Other explanation for relating bacteria with such malignancy, in general, bacteria are able to produce a wide range of carcinogens, mutagens or tumor promoters from a wide range of substance such as Tryptophan metabolites, Volatile phenols, and N-nitroso compounds. Many bacteria present in the urine reduce diet-derived nitrate to nitrite, which under mildly acidic or neutral conditions becomes a potent nitrosating agent. The production of *N*-nitrosamines by the nitrosation of amine precursors was detected in the urine of bacterially infected rats 27). Therefore, in addition to the *N*-nitrosamine exposure originating from the external environment, individuals with bacterial cystitis are potentially more exposed to nitrate and/or nitrite, which would then greatly increase the risk of in situ formation of carcinogenic alkylating agents, e.g., *N*-nitrosamine. Nitrosamines are capable of inducing bladder cancer in animal model(28). Gram negative – bacteria were able to produce nitosoamine compounds as a potent carcinogenic agent, in the urine of patients with bladder cancer(29).

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