Enhancement of Urinary Bladder Carcinogenesis by the Role of Chronic Bacterial Infection-induced Inflammation (Immunohistochemical and Biochemical studies)

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Abstract

Background: Bacterial infections traditionally have not been considered major causes of cancer. Recently, however, bacteria have been linked to cancer by two mechanisms: induction of chronic inflammation and production of carcinogenic bacterial metabolites. The most specific example of the inflammatory mechanism of carcinogenesis is *Escherichia coli* infection. *E. coli* has been epidemiologically linked to urothelial carcinoma of the urinary bladder by its propensity to cause lifelong inflammation. This inflammation is in turn thought to cause cancer by inducing cell proliferation and production of mutagenic free radicals and N-nitroso compounds.

Material and methods: After each 3, 6 and 9 months of daily oral administration of dibutyl amine (DBA) plus sodium nitrate (nitrosamine precursors) in drinking water, curcuma in grinding diet and bladder injection with *E. coli*, rats were sacrificed. The excited bladder were dissected, processed and stained with H&E and anti-Ki67 immunohistochemical stains. This was followed by Elisa for caspse-3 and statistical analysis.

Results: The current results indicated that *E. coli* infection in the bladder tissues increases the carcinogenic ability of nitrosamine precursors through caused marked alteration in the form hyperplastic, dysplastic and metaplastic urothelium. Also, there was a statistically significant increase in ki67 immunoreactivity in urothelium. However, a statistically significant decrease in the concentration of caspase-3 in bladder tissue consequently caused the process of carcinogenesis. All these changes were less marked after curcuma treatment when compared with the group that not treated with curcuma.

Conclusion: Bacterial infection of the urinary bladder may play a major additive and possible role in bladder carcinogenesis. Rhizome of curcuma may have a protective action during induction of urinary bladder tumors.

Keywords: Bladder carcinogenesis - *E. coli* - curcuma – DBA - Ki67-Immunohistochemistry - Caspase-3.

Introduction

Bladder carcinoma is the most common malignancy of the urinary tract (Ploeg *et al.*, 2009), where the cells lining the urinary bladder lose the ability to regulate their growth and start dividing uncontrollably. This abnormal growth results in a mass of cells that form a tumor (Good, 2003). Urothelial carcinomas account for more than 90% of urinary bladder cancer cases.
Enhancement of Urinary Bladder Carcinogenesis….

(Hwa-Chain et al., 2011), and about 4% of all malignant tumors and approximately 7% of all urinary tract malignancies (WHO, 1997). Bladder cancer affecting the urinary tract worldwide and it accounted for 386,000 cases and 150,000 deaths in 2008 (Jemal et al., 2011). In Egypt, carcinoma of the bladder is the most prevalent cancer and account 30.3% of all cancers at the National Cancer Institute (NCI), Cairo (Khaled et al., 2004). The incidence of the bladder cancer in Egypt was 26 / million / year in males and 7 / million / year for females (Mokhtar, 1991).

Uropathogenic *Escherichia coli* (UPEC) are responsible for approximately 90% of urinary tract infections (UTI) (Todar, 2007). Caspases, free radicals and other proteins have been included in the pathogenesis of many diseases (Choudhary and Wang, 2009). Caspases are a group of cysteine proteases that cleave after an aspartic acid residue of a specific recognition site. This extrinsic activation then triggers the hallmark caspase cascade characteristic of the apoptotic pathway (Alnemri et al., 1996). Caspase-3 is a major effectors caspase that plays a critical role in the apoptotic cascade (Porter, 1999).

On the other hand, the Ki67 is a molecule that can be easily detected in growing cells in order to gain an understanding of the rate at which the cells within a tumor are growing, where Ki67 has been suggested as having prognostic value in renal cell carcinoma and urothelial neoplasms of the urinary bladder (Yurakh et al., 2006). This 345 kDa protein is present in proliferating cells in all cell-cycle phases (G1, S, G2 and M) and absent in quiescent cells (G0); thus, it can be used as a marker of growth fraction (Gerdes et al., 1984).

**Aim of the study:**
The present work was designed to study the possible role of chronic inflammation induced by *E. coli* in the urinary bladder of rats, the protective role of curcuma, in addition to the early detection for ki67 (as proliferative index) and caspase-3 (apoptotic modulator) during bladder carcinogenesis.

**Material and Methods**

**Material**

1- **Chemicals**
Dibutylamine \([\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}]\), is a colorless liquid with ammonia odor and molecular weight of 129.244 gm/mol. and Sodium nitrate \([\text{NaNO}_3]\), is a white powder or colorless crystals with sweet smell were purchased from BDH ltd pool (England) and Arabic Laboratory Equipment Co. (Egypt) respectively. All other chemicals were of the highest purity commercially available.

2- **The natural product (curcuma)**
Curcuma was purchased from shop perfumery and was identified by Medicinal and Aromatic Plants Department, National Research Centre, Giza, Egypt.

3- **Microorganism *E. coli***
*E. coli* originally isolated from the urine of patients with urinary tract infection (Nasser Institute, Cairo, Egypt). The *E. coli* were grown overnight in brain-heart infusion broth (BHI broth) medium then heat-killed in boiling water
for 30 min. The suspension was centrifuged and the sediment was suspended in phosphate buffered saline at a density 2 x 10^6 cells/ml and stored in 30-ml portions at -20°C until used. Once the suspension was thawed, any portion remaining after use was discarded (Yamamoto et al., 1992).

4- Experimental Animals and Dosing

The experiment was conducted in accordance with the provisions of the guide for care and use of laboratory animals (NIH, 1985). One hundred and fifty adult male albino rats, each weighing 60-80 gm. were kept in the laboratory under constant conditions of temperature 25°C, and were maintained on a standard diet and water *ad libitum* for at least one week before the experimental study.

The experimental rats were divided into five groups as follow:

**Group 1:** Fed on the basal diet and normal water and left in normal condition, this group was served as control group.

**Group 2:** Bladder injected with 2 x 10^6 organisms suspended in 0.1 ml of phosphate-buffered in 2.1% NaCl solution (Yamamoto et al., 1992). This group was served as *E. coli* group.

**Group 3:** Administered drinking tap water containing 1.32 ml/L DBA and 2 gm/L sodium nitrate (El Gendy et al., 2007). This group was served as nitrosamine precursors group.

**Group 4:** Administered drinking tap water containing 1.32 ml/L DBA and 2 gm/L sodium nitrate and the bladder was injected with 2 x 10^6 *E. coli* bacteria in 0.1 ml salin. This group was served as *E. coli* and nitrosamine precursors group.

**Group 5:** Fed normal grinding diet supplemented with 1% of curcuma, drink tap water containing 1.32 ml/L DBA and 2 gm/L sodium nitrate and bladder was injected with 2 x 10^6 *E. Coli* bacteria in 0.1 ml saline. This group was served as *E. coli* plus nitrosamine precursors and curcuma group.

Diet was prepared three times a week just before the feeding time. DBA and sodium nitrate were mixed with the drinking water using magnetic stirrer for a period of 20 min.

**Methods:**

At the end of the experimental period, animals were sacrificed and the whole bladder was removed, washed with saline, dried, cut into weighted pieces and frozen -80°C until used. Bladders were obtained to carry out histopathological and immunohistochemical studies. Body weight of rats in each group was recorded at the beginning of the experiment and at the end of each stage of scarification.

1- Histopathological findings:

For histopathological studies, bladder tissue pieces were fixed in 10% formalin, blocked in paraffin, sectioned (4-6 µm) and stained with hematoxyline and eosin according to Banchroft et al. (1996).

2- Immunohistochemical observations:

Sections of bladder fiwere immunostained using study anti Ki67 primary antibody for 60 minutes, followed by the secondary antibody application using labeled streptavidine –biotin. Sections were counterstained with hematoxyline
Bancroft and Gamble, 2002) and analyzed by image analyzer for calculation of Ki67 labeling index (Ki67 LI). The results were expressed as mean ± standard error (Ki67 LI = mean ± SE %) using SPSS 11 for windows.

2.1 Immunoreactivity scoring

The sections were screened for positive cells, defined as cells with nuclear staining. The amount of Ki67 staining was scored in percentages. The areas with maximal immunohistochemical staining were used for scoring. A total, 300-500 cells were scored. In the visual estimation, only definitely brown nuclei were recorded as positive. The results were expressed as percentage of immunoreactive infected cell nuclei (Antoine et al., 2007).

3- Biochemical measurements

Total protein was determined according to Gornall et al. (1949). Caspase-3 concentration was determined using the CaspACE™ Assay System, Colorimetric kit according to the manufacturer’s instructions.

4- Statistical analysis

All data were expressed as the mean ± standard error (SEM). The statistical significance of differences among means was assessed by the one way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. Survival analysis was utilized to analyze the survivability of all animal groups. Differences between two groups were assessed by student's t test. Statistical differences were considered significant at p≤ 0.05. The least significant difference (LSD) test was used to separate the mean values according to Steel and Torrie (1981).

Results

A- Histopathological findings

Bladder histopathological changes due to bacterial infection, nitrosamine precursors and therapeutics effect of curcuma are shown in (fig.1). Examination of H and E sections of the urinary bladder of the control rats showed normal histological structure that consisted of the lining mucosal epithelium (transitional epithelium) since intermediate between non keratinizing squamous and pseudostratified columnar epithelium that about 5-7 cell layers, covered by superficial urothelium that is single layer of umbrella cells with eosinophilic cytoplasm and prominent nucleoli, while Intermediate urothelial cells are cuboidal to low columnar with well defined borders with ovoid nuclei, basal urothelial cells are more cylindrical and longitudinal nuclear grooves. The urothelium with underlying lamina propria is thin, contains loose to dense connective tissue, blood vessels, lymphatic, adipose tissue and muscularis that consists of longitudinal and circular muscle bundles. In this group there was no histopathological alteration during all treatment periods (Fig. A -1). In E coli group there were focal stratification and hyperplasia in the lining mucosal epithelium, maintains morphologic evidence of maturation from basal cells to superficial cells, absence of cytologic atypical, while the underlying lamina propria had sever congestion in the blood capillaries.
with inflammatory cells infiltration (Fig. B -1). In nitrosamine precursors group, dysplasia appeared in the mucosal lining epithelium that characterized by lack of maturation in basal and intermediate cell layers (not full thickness). Urothelial thickness (may be increased or decreased) and superficial umbrella cells were present. Atypical cytological changes were restricted to intermediate and basal cells, loss of cytoplasmic clearing and nuclear polarity, nuclear enlargement, nuclear membrane irregularities and nuclear hyperchromasia, while the underlying lamina propria showed congestion in newly formed capillaries (Fig. C -1). In nitrosamine precursors plus E. coli group, the serosal layer showed granuloma like formation is a tiny collection of macrophages in which immune system attempts to wall off substances that it perceives as foreign but is unable to eliminate. Such substances include infectious organisms such as bacteria with inflammatory cells infiltration (Fig. D -1). In nitrosamine precursors plus E. coli group and protected with curcuma, showed normal bladder layers except congestion in the blood capillaries in the lamina propria (Fig. E -1).

B- Immunohistochemical observations

Urothelium of control and curcuma treated rats stained with immunostained for Ki67 showed few weak positive stained nuclei indicating the cell division of some urothelial cells (Figs. 2A and 2E). However, most of the urothelium showed strongly positive stained nuclei in rats treated with E. coli and nitrosamine precursors plus E. coli (Figs. 2B and 2D). Also, the urothelium of rats treated with nitrosamine precursors plus E. coli and curcuma illustrated that, the positively stained nuclei were markedly decreased approximated control section from that of nitrosamine precursors plus E. coli treated rats (Fig. 2E).

As shown in Table 2 and fig. 3, the effect of nitrosamine precursors, E. coli and curcuma on urinary bladder ki67 proliferating index were presented, and the analysis of variance revealed that, there was a significant difference between different treatment groups. Nitrosamine precursors plus E. coli treated rats and E. coli treated rats after 9 months illustrated a significant increase in urinary bladder Ki67 labeling index. However, rats protected with curcuma and treated with nitrosamine precursors plus E. coli showed a high significant decrease and depletion in the number of Ki 67 positive reaction in all layers of the epithelium with respect to control group and very high significant decrease when compared with nitrosamine precursors plus E. coli treated animals.

C- Detection of caspase-3

As shown in table (2), the present results indicated that, a comparison between mean level of caspase-3 concentrations in groups showed that, all treated groups were recorded lower values than control during the first 3 months, lowest values in nitrosamine precursors plus E. coli group and followed by the group that protected by curcuma. During 6 and 9 months, nitrosamine precursors plus E. coli group was recorded also the lowest values, while the group
that protected with curcuma started in increasing nitrosamine precursors plus E. coli group. of caspase-3 concentrations when compared to

Table (1): Urinary bladder Ki67 labeling index as a result of carcinogenicity of E. coli, nitrosamine precursor and curcuma administration during different intervals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Intervals</th>
<th>3 months (%)</th>
<th>6 months (%)</th>
<th>9 months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.22 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.22 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.22 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>4.44 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.53 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.02 ± 2.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrosamine precursors</td>
<td>5.02 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.61 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.25 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrosamine precursors plus E. coli</td>
<td>13.91±1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.63 ± 0.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.75 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrosamine precursors plus E. coli plus curcuma</td>
<td>5.6 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.60 ± 0.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.07 ± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>F value</td>
<td>35.48531</td>
<td>108.7714</td>
<td>25.61225</td>
<td></td>
</tr>
<tr>
<td>F crit</td>
<td>2.578739</td>
<td>2.578739</td>
<td>2.578739</td>
<td></td>
</tr>
</tbody>
</table>

Within each column, means superscript with different letters are significantly different (P≤ 0.05).

Table (2): Effect of E. coli, nitrosamine precursor and curcuma administration on caspase-3 concentrations of bladder tissues during different intervals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Intervals</th>
<th>3 months (μM)</th>
<th>6 months (μM)</th>
<th>9 months (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.1 ± 2.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.17 ± 2.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.17 ± 2.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>61.5 ± 6.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.67 ± 18.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.67 ± 8.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrosamine precursors</td>
<td>34.83±3.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31 ± 6.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.5 ± 0.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrosamine precursors plus E. coli</td>
<td>20.5 ± 2.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.67 ± 0.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.83 ± 1.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Nitrosamine precursors plus E. coli plus curcuma</td>
<td>28 ± 1.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.3 ± 4.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34 ± 3.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>F value</td>
<td>74.77341</td>
<td>13.95873</td>
<td>89.09777</td>
<td></td>
</tr>
<tr>
<td>F crit</td>
<td>2.75871</td>
<td>2.75871</td>
<td>2.75871</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1): Light micrograph showing (A) normal bladder tissues in control gp. (B) hyperplasia, there is an increase in the number of urothelial layers (more than 7 layers) and stratified urothelium in *E coli* gp. (C) showing dysplasia, consists of an expansion of immature cells, with a corresponding decrease in the number and location of mature cells in the mucosal lining epithelium (s) in nitrosamine precursors gp. (D) granuloma formation (g), is a tiny collection of macrophages in which immune system attempts to wall off substances that it perceives as foreign but is unable to eliminate. Such substances include infectious organisms such as bacteria with inflammatory cells infiltration (m) in serosal layer showed in nitrosamine precursors plus *E. coli* gp. (E) normal bladder layers except congestion in blood capillaries of lamina propria (v) in nitrosamine precursors plus *E. coli* plus curcuma gp. (H and E, X40)
Enhancement of Urinary Bladder Carcinogenesis….

**Discussion**

An experimental study in rats had shown associations between chronic infections with *Escherichia coli* and early bladder neoplasia (Davis et al., 1991). Several studies concluded that urinary tract infection

**Figure 2**: Immunostaining micrograph of Ki67 expression in different groups (the positivity is brown nuclear staining). Control (A), *E. coli* gp (B), nitrosamine precursors gp (C), nitrosamine precursors plus *E. coli* gp (D) and nitrosamine precursors plus *E. coli* gp treated with roots of curcuma (E). (Labeled Streptavidin Biotin, X 200).
Discussion

An experimental study in rats had shown associations between chronic infections with Escherichia coli and early bladder neoplasia (Davis et al., 1991). Several studies concluded that, urinary tract infection promotes urinary tract carcinogenesis in rat and infection with Escherichia coli produced permanent infection, acute and chronic inflammation and urothelial hyperplasia (Schetter et al., 2010).

The results in the current study indicated that, E. coli infection in the bladder tissues increased the carcinogenity of nitrosamine precursors which is in agreement with Ashmawey et al. (2011). This may be due to the increase of production of nitrite and nitrosamine by E. coli. These results are also in agreement with Rex et al. (2008), who found that, N-butyl-N-(4-hydroxybutyl) nitrosamine has a strong ability to induce rat bladder cancer. The present study showed that, bacterial infection of the urinary bladder may play a major role in bladder carcinogenesis, both by helping in-situ nitrosamine synthesis and by augmenting carcinogenesis by nitrosamines. This result may agree with Jhamb et al. (2007) who stated that, when animals receiving amine and nitrate, indicating re-absorption of the carcinogen synthesized in situ to induce distant organ transformation. In another case, E. coli infection augmented bladder carcinogenesis by N-nitrosobutyl (4-hydroxybutyl) amine (NBHBA) as indicated by earlier appearance of bladder tumours.

Some mechanisms were suggested for the tumor enhancing effect of E. coli induced inflammation in the present experiment. Firstly, the formation of the intracellular bacterial communities (IBC), secondly, Uropathogenic (UPEC) colonization is counteracted by the innate host responses to infection, which include soluble defensive molecules, cytokine secretion and neutrophil influx which increased bladder inflammation and polymorphonuclear cell (PMN) influx in response to the proinflammatory cytokine, interleukin 6 (IL-6) and the PMN chemo tactic cytokine, IL-8, respectively. This host inflammatory response is augmented by E. coli invasion of urothelial cells (Schilling et al., 2001). Tissue damage caused by the influx of neutrophils, as well as the generation of secreted toxins by UPEC, also likely facilitate bacterial dissemination throughout the multiple layers of bladder epithelium (Higgs and Pollard, 2000). Thirdly, the initiation of bladder carcinogenesis could be due to the nitrosation of secondary amines with ingested or metabolically derived nitrite that leads to N-nitrosamine formation (Hill, 1988).

In the present study, the Ki67 index is a useful marker of cell proliferation and was significantly greater in rats that received nitrosamine precursors and E. coli. These results agree with El-kott (2007) who reported that, urinary bladder Ki67 labeling index was very high in N-methyl-N-nitrosourea (MNU) precursors group when compared with control rats. However, rats which were protected with
garlic extract and treated with N-MNU precursors showed a high significant increase with respect to control group and very high significant decrease when compared with MNU precursors treated animals. the present results were also in agreement with Tsuji et al. (1997) who concluded that, immunohistochemical analysis for Ki67 are useful prognostic indicators in patients with urinary bladder cancer who undergo radical cystectomy.

The present findings have shown that *E. coli* infection was associated with a significant decrease in the concentration of caspase-3 protein, one form of mediators of apoptosis pathways. However, it is significant that tumors did not develop in the groups treated with *E. coli* plus carcinogen. This suggests that, inflammatory reaction by *E. coli* only is insufficient to induce tumors but may be sufficient to augment neoplastic changes induced by carcinogen.

The current results have also demonstrated the presence of caspases-3 protein in bladder tissues but it was down regulated than that found in control group and this may be due to functional deletion or mutation in the caspases-3 gene (Yang et al., 2001).This results is in agreement with Burton et al.(1999) who suggested that, the measurement of caspase 3 levels may be useful in monitoring patients with bladder carcinoma in situ, to identify both those at increased risk for invasive bladder cancer and those who are more likely to have a response to intravesicular immunotherapy. The current results were also in agreement with another reports by Karamitopoulou et al. (2010). The decreased detection of active caspase-3 carcinomas suggested that, alterations in interrelated apoptosis markers may play an important role in the progression of urothelial carcinoma from a superficially infiltrating to a muscle invading tumour and would help to better characterize a subpopulation of T1 carcinomas that could profit from early cystectomy or more aggressive adjuvant chemotherapy. Active caspase-3 might be an important prognostic factor in bladder cancer.

In the current study, curcuma treated group showed significant decrease in the reduced caspase-3 level. This result indicated the ability of curcuma with its rich anti carcinogens substance to protect bladder from sever carcinogenic action of nitrosamine and minimize its effect. The present findings were in agreement with Katia et al. (2009) who reported that, Curcuma could be an option for treatment of bladder cancer and with Kawamori et al. (1999) who concluded that, chemopreventive activity of curcuma has been indicated when administered before, during and after carcinogenic treatment as well as when administered during the promotion and progression phase of colon carcinogenesis in rats. Also the present results were in concomitant with Tian et al. (2008) who stated that, curcuma could prove an effective chemopreventive and chemotherapy agent for bladder cancer, in vivo study revealed curcuma did induce apoptosis in situ, inhibit and slow the development of bladder cancer, where exposure
of human bladder cancer cells to curcuma resulted in the induction of apoptotic cell death and caused cells to arrest in the G2/M phase. At the same time, curcuma treatment did not cause any side-effect.

References:
Enhancement of Urinary Bladder Carcinogenesis....


Enhancement of Urinary Bladder Carcinogenesis

(Study of Histochemistry and Biochemistry)

Mohamed Sayed Gabr*, Ayman Eldredin Ibrahim*, Ralph Majd Hsany*,
Mohamed Saeed Gabr, Ayman Eldredin Ibrahim,
Rahaf Majd Hsany, Mohamed Saeed Gabr

The infection by bacterial inflammation is one of the common causes of bladder cancer. Recently, some studies have linked the bacteria to the occurrence of cancer through two mechanisms: the development of chronic inflammation and the production of substances that cause cancer.

Chronic inflammation due to bacterial infection, especially with E. coli, is one of the causes of bladder cancer. This study aimed to investigate the role of bacterial infections causing chronic bladder infections in the occurrence of bladder cancer through injecting the bladder of adult male rats with E. coli bacteria and giving them daily doses of nitrosylamino and sodium nitrate (salts of nitrosamines) dissolved in water and turmeric as a chemical protective agent to evaluate its therapeutic capacity during the occurrence of bladder cancer for nine months, collecting samples every three months through the sexual examination of the bladder and preparing it for histological and histochemical studies using hematoxylin-eosin and immunohistochemical staining against Ki-67, followed by analysis of the images using a photo analyzer to calculate the tumor index and after that performed the EIA for the detection of anti-3-caspsin protein in bladder tissues and statistical analysis.

This study showed that the bacterial infection of the bladder had increased the capacity for bladder cancer and caused noticeable changes in the epithelial layer (lining layer of the bladder wall) through cell growth and change in the shape of the nucleus and the appearance of new cells. In addition, there was a statistically significant change in the immunohistochemical staining against Ki-67 antigen inside the nuclei of those cells. And this study also showed a decrease in the caspase-3 protein in the bladder tissues, especially in the group that was not treated with turmeric compared to the control group.

From what has been mentioned, we can conclude that the bacterial infection of the bladder plays a major role in the process of bladder cancer and turmeric plays a protective role in the process of cancer. And both Ki-67 and caspase-3 are indicators of cancer that help in the early detection of this disease.