

Investigation of Genotoxic Effect of Escherichia coli in Urinary Tract Epithelial Cells with Micronucleus Assay

Escherichia coli'nin Üriner Sistem Epitelyal Hücrelerinde Mikronükleus Testi ile Genotoksik Etkisinin İncelenmesi

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Öz

Amaç: E. coli, kadınlarda idrar yolu enfeksiyonlarına neden olan bir bakteri türüdür. Bu çalışmada kadınların idrar yolu epitel hücrelerinde, enfeksiyona bağlı DNA hasar etkilerinin araştırılması amaçlandı. **Metot:** 40 E. coli enfeksiyonu olan ve 40 enfeksiyon olmayan kadınların idrar yolu epitel hücreleri toplandı ve mikronükleus analizine tabi tutuldu. Her iki grupta mikronükleus ve binükleus parametreleri genom stabilitesinin değerlendirilmesi için kullanıldı. Tüm idrar örneklerinin pH, lökosit, lökosit esteraz, nitrit değerleri H-800 ve FUS-200 tam otomatik idrar cihazı ile analiz edildi. **Bulgular:** E. coli enfeksiyonu olan ve enfeksiyon olmayan grupların sonuçları istatistiksel olarak Mann-Whitney U ve Independent Sample T testi ile değerlendirildi. Mikronükleus, binükleus, idrar, lökosit, lökosit esteraz ve nitrit parametreleri için sonuçlar istatistiksel olarak anlamlı bulundu ($p<0,05$). pH sonuçları ise istatistiksel olarak anlamlı bulunmadı ($p>0,05$).

Sonuç: İdrar yolu epitel hücrelerinde bulunan E. coli' nin, DNA hasarını indüklediği görülmüştür. Antibiyotik tedavisinin hastalarda erken kullanımı ve uygun dozda kullanımı ile DNA hasar seviyesini azaltmaya yardımcı olabileceği söylenebilir.

Anahtar Kelimeler: Mikronükleus, İdrar yolu, DNA hasarı, Escherichia coli

Abstract

Object: E. coli is a type of bacterium that causes urinary tract infections in women. The aim of the work was to investigate the possible use of epithelial cells from the urinary tract in identifying infections-related DNA damage effects in women. **Methods:** Epithelial cells from urinary tract were collected from 40 E. coli infection and 40 control (non-infection) women and subjected to micronucleus assay. Number of cells with micronucleated, binucleated cells were counted in two groups as parameters for the evaluation of genome stability. All of urine samples pH, leukocyte, leukocyte esterase, nitrite results measured with H-800 urine analyzer and FUS-200 urine sediment analyzer. **Result:** E. coli infection and non-infection groups test results were statistically evaluated with Mann-Whitney U and Independent Sample T test. A p-value of <0.05 was found statistically significant for micronucleus, binucleus, and urine, leukocyte, leukocyte esterase and nitrite parameters. pH results was not found statistically significant ($p>0,05$). **Conclusion:** The detection of E. coli in urinary tract epithelial cells showed that induce DNA damage. It can

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be said that patients with early antibiotics and doses, early treatment may help to reduce the level of DNA damage.

Keywords: Micronucleus, Urinary tract, DNA damage, Escherichia coli

Introduction

Urinary tract infections (UTIs) include upper and lower urinary tract infections in which urothelial infections occur by the proliferation of pathogens. Urinary tract infections are the most common extraintestinal diseases among women in the world, and they cause diseases such as urethritis, cystitis and acute/chronic pyelonephritis [1,2]. Women are at the risk of UTIs because of they have urine retention, sexual activity and spermicides as well as short urethra [3]. Commonly *E. coli* strains, found in the intestine, have symptoms of urinary tract infections when they enter the urinary tract [4]. *E. coli* strains synthesize another genotoxin, colibactin which natural products produced. The colibactin (clb) -like metabolites are encoded by a hybrid polyketide synthase (pks), non-ribosomal peptide synthetase (nrps) gene cluster termed clb or pks. The *E. coli* strains are divided into the main groups A, B1, B2, D, and E. The B2 phylogroup has mainly the pks genomic island. pks+ *E. coli* strains induce DNA damage in eukaryotic cells [5,6].

In the present study, We investigated the genotoxic effect of *E. coli* by micronucleus assay in women with urinary tract infection.

Materials and Methods

We selected 2 group with *E. coli* infection and control (non-infection) female at Süleyman Demirel University Hospital (Isparta, Turkey) between August 2017 and October 2017.

A total of 80 individuals consisting of 40 *E. coli* infection, and 40 control female patients were monitored for DNA damage in urinary tract epithelial cells by Micronucleus assay. The age of women participating in the study varies between 19 and 75 years. All of the 80 urine samples were analysis with dipstick for pH, nitrites, leukocyte esterase. Leukocyte count detected with automatic urine sediment analyzer.

After evaluating the urine microscope, samples of detected epithelial cells were cultured in bacterial cultu-

re bloody and Eosin Methylene Blue (EMB) agar. If bacterial culture include to *E. coli*, the samples were evaluated as patient group. If bacterial culture not included to *E. coli*, the samples were evaluated control group (Figure 1).

Epithelial cells were isolated from urine samples by centrifugation and slides prepared according the method previously described by Lehucher-Michel et al. [7]. In order to minimize degenerative cell changes, urine samples were collected in sterile plastic bottles and stored at 4°C. Epithelial cells from the urinary bladder and ureter were concentrated by 10 min centrifugation (400X g). Urine was removed and fixed in 5 ml of fresh fixative solution (methanol/acetic acid, (3 : 1). Following a 20 min storage period at 4°C cells were submitted to a second 10 min centrifugation (400 X g). The fixative solution was not completely removed and the pellet was resuspended and dropped onto pre-cleaned microscope slides and dried for 24 h at room temperature. The samples were collected by asking age, antibiotic, pathological information, exposure to radiation and chemical agents. The Ethical Committee of Süleyman Demirel University approved this study (Ethics committee No:122).

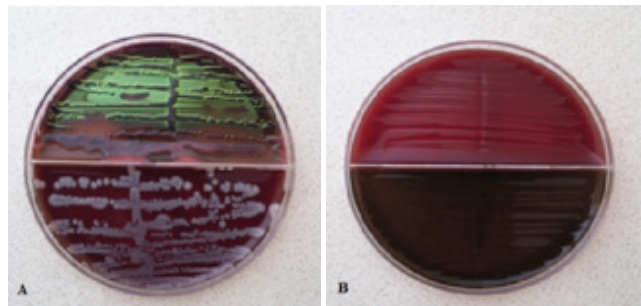


Figure 1.

E. coli colonies (green metallic sheen) on EMB agar (A). Not colonize *E. coli* (B).

Collection of Urine Samples and Personal Information

Only women patient samples were taken from each patient using sterile urine container. The patients were asked if they have any chronic and hereditary disease, antibiotic use at least 1 month and exposure to radiation/chemical agents.

Evaluation of Micronucleus Assay and Urine Dipstick

All of urine samples pH, leukocyte, leukocyte esterase, nitrite results measured with H-800 urine analyzer and FUS-200 urine sediment analyzer. Urinary tract epithelial cells was stained according to the method of Papanicolaou 1963 [8] with some modifications. Micronucleus (MN), binucleus (BN) parameters have been evaluated with 400X magnification of the light microscope. For each slides, 1000 cells were examined.

Statistical Analysis

IBM SPSS 24 program was used for statistical evaluation. Mann-Whitney U test was performed to compare the number of cells micronucleus, binucleus, urine pH, leukocyte and nitrite parameters. Independent sample t test was performed to compare the leukocyte esterase. Mean with SD's for each groups were calculated.

Results

In our study, DNA damage in urinary tract epithelial cells in 40 patients and 40 control group was investigated by micronucleus assay. Normal epithelial cell, micronucleated and binucleated cells displayed Figure 2.

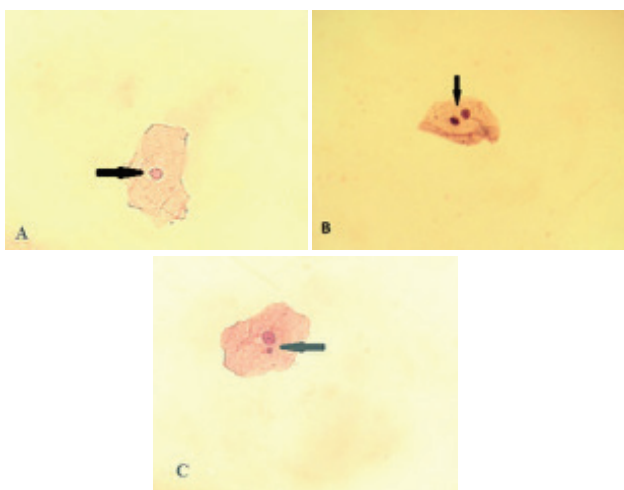


Figure 2. Normal urinary tract epithelial cell with Papanicolaou stain demonstrated (A), binucleus (B), micronucleus (C). Magnification: 400X.

We found 17,5% binucleus, 30% micronucleus and 52,5% normal epithelial cells in infection group, respectively. However, non-infection group we found 5% binucleus, 2,5% micronucleus and 92,5% normal, respectively (Figure 3). Micronucleus, binucleus regarded as statistically significant ($p < 0.05$) (Table 1).

Table 1 Micronucleus, binucleus statistical results

Mean \pm Std. Error	P
E. coli infection group	
Micronucleus	0.28 \pm 0.124 <0.05
Binucleus	0.20 \pm 0.064 <0.05
Non-infection group	
Micronucleus	0.03 \pm 0.025 <0.05
Binucleus	0.05 \pm 0.035 <0.05

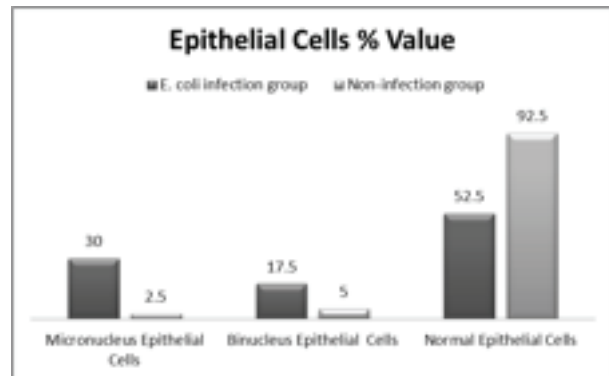


Figure 3. Epithelial cells % value

As a result of chemical analysis performed in control and patient group, leukocyte leukocyte esterase and nitrite results were found to be statistically significant ($p < 0.05$). Only urine pH result not statistically significant ($p > 0.05$). Urine analyze pH, leukocyte leukocyte esterase, nitrite results shown in Table 2.

Table 2 Comparison of urine analyze test results

Urine Analyze	pH	Leukocyte	Leukocyte Esterase		Nitrite	
	(Mean/Std.Error)	(Mean/Std.Error)	Pos	Neg	Pos	Neg
E. coli infection	6,03±0,08	137,4±41,73	32	8	15	25
(n=40)			(%80)	(%20)	(%37,5)	(%62,5)
Non-infection	5,85±0,12	30,87±56,36	23	17	0	40
(n=40)			(%57,5)	(%42,5)		(%100)

Discussion

The aim of this study was to evaluate DNA damage in urinary tract epithelial cells with Micronucleus Assay. *E. coli* plays an important role in the diagnosis of urinary tract infection, affecting 150 million people each year worldwide [9]. Recurrent urinary tract infections (UTIs) are among the most common bacterial infections, especially in the women.

Khan et al [10] studied urinary tract infection and associated risk factors in post-menopausal women. They found the prevalence of UTIs was found to be 15.7% and common clinical condition affecting the ageing women. Testing for the presence of micro-organisms in the urinary tract, in order to diagnose asymptomatic bacteriuria or symptomatic urinary tract infections (UTI), is very common at all levels of health care [11].

Several rapid screening tests are used commonly to make a presumptive diagnosis of UTI, including dipstick biochemical analysis of urine for pH, nitrites, leukocyte esterase. The leukocyte esterase dipstick a widely used to identify patients with pyuria associated with infection [12-13]. Devillé et al [11] studied with the urine dipstick test to rule out infections. Overall, their research demonstrates that the urine dipstick test alone seems to be useful in all populations to exclude the presence of infection if the results of both nitrites and leukocyte-esterase are negative. Urinary system infections support with urine pH of 7.5 and above a study by Özer et al [14] of 575 patients with leukocyte esterase, leukocyte, nitrites results was

found significantly related with urine culture results but urinary tract infections reported was not statistically significant between urine pH and UTIs. Thornton et al [15] studied to determine the effect of different pH and urine concentrations on *E. coli* growth in vitro. The lowest log CFU/mL were observed in alkaline concentrated urine. According the present study *E. coli* infection and non-infection cases leukocyte esterase, leukocyte and nitrites results was found statistically significant ($p < 0.05$) but pH was not statistically significant ($p > 0.05$).

E. coli is the most common cause of infections by Gram-negative bacilli and induces DNA double-strand breaks in eukaryotic cells [16]. In assessing chromosomal damage, the MN test is used as a marker of genomic instability in the standard genotoxicity system [17]. Cuevas-Ramos et al [18]. show that a single, short exposure of cultured mammalian epithelial cells to live pks+ *E. coli* at low infectious doses induced a transient DNA damage response followed by cell division with signs of incomplete DNA repair, leading to anaphase bridges and chromosome aberrations. Micronuclei, aneuploidy, ring chromosomes, and anaphase bridges persisted in dividing cells up to 21 d after infection, indicating occurrence of breakage–fusion–bridge cycles and chromosomal instability. In this study *E. coli* infection and non-infection cases MN and BN results was found statistically significant ($p < 0.05$).

The application of the micronucleus test to epithelial cells of various human tissues will provide evidence of exposure to carcinogens and clastogens. The de-

tection of E. coli in urine, early antibiotics and doses, early treatment may help to reduce the level of DNA damage.

Disclosure Statement

The authors of this study have no relevant financial interests to report.

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