BRIEF REPORT

IMMUNOLOGICAL AND BIOCHEMICAL RESPONSE FROM OLDER ADULTS WITH URINARY TRACT INFECTION TO UROPATHOGENIC *Escherichia coli* VIRULENCE FACTORS

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ABSTRACT

Descriptive study in which 24 urine samples from older adults with urinary tract infection (UTI), from nursing homes, were evaluated; in order to identify differences in the immune and biochemical response from older adults with UTI by *Escherichia coli* (*E. coli*) to major virulence factors in the pathogenesis of UTI. Iron concentration, TNF-α, IL-1β and antioxidant capacity in urine were determined. A relation was found between, an increase in iron and red blood cell concentration in urine, and the presence of the *pap GII* gene found in *E. coli.* It is concluded that older adults, with UTIs by *E. coli* with the gene *pap GII*, have increased tissue damage.

Keywords: Nursing Homes; Elderly; Uropathogenic *Escherichia coli*; Biomarkers; Virulence Factors, Urinary Tract Infections, Immunology, Urine, Antioxidants, Cytokines (Source: MeSH NLM).

INTRODUCTION

Escherichia coli (E. coli) is the most frequent cause of bacteremia in men and women [1], and the urinary tract is the main way of infection in geriatric patients [2]. This is partially due to the fact that the older adult population (OAP) presents particular immunological characteristics, and a subclinical state of chronic inflammation, known as immunosenescence, where the polymorphonuclear lineage (main line of defense in urinary tract infections [UTIs]) [3] has limited function [4]. Several *E. coli* virulence factors have been described, mainly associated with bacteremia and sepsis, which include cell adhesion molecules, iron uptake systems and exotoxins that form a protein system that allows the bacteria to elude or injure the patient's immune system [5,6].

The interaction between the immune system of patients with UTIs and the genetic ability of the bacteria to form virulence factors determines the bacterial clearance in the urinary tract [6], so it is necessary to explore the difference in the immune and biochemical response of older adults with UTIs to the various virulence factors associated with sepsis in uropathogenic E. coli (UPEC).

THE STUDY

Between April and July 2018, the urine of 24 older adults with UTIs, of both sexes, residing in private gerontological nursing homes in Lima, was evaluated. The diagnostic criteria for

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UTIs were defined as: a microscopic count of >5 leukocytes per field of 400x magnification, the conversion of nitrites by the Griess method and a count of more than 100,000 colony-forming units in the blood agar culture medium. Urine sediment analysis was standardized according to the recommendations of the Chilean Health Institute [7].

The etiological agent was identified by using traditional biochemical methods and the bacteria identified as *E. coli* were preserved in tryptic soy broth and 20% glycerol. Polymicrobial cultures were excluded from the study. Subsequently, the bacterial DNA was extracted using the GeneJet Genomic kit (Thermo Scientific®, Massachusetts, USA) and the presence of 11 virulence genes was evaluated: *aer, α-hly, cnf-1, sfa, chuA, TcpC, nanA, pap GI, GII, GIII* and *iucC* by end-point polymerase chain reaction.

The urine samples were centrifuged at 3,000 g (Sigma, 3-30KS) for 10 minutes, preserving the supernatant. Concentrations of TNF-α, IL-1β and iron were determined; besides, the total antioxidant capacity in urine was evaluated by ABTS•+ and FRAP methods, which acted as immune response markers through the increase of reactive oxygen substances (ROS). All analytes were measured using the Multiskan Go spectrophotometer (Thermo Scientific®, Massachusetts, USA).

The statistical analysis was carried out with the Epidat version 4.1 program. The description of the qualitative variables was made through frequency tables. Shapiro Wilk's normality test was applied to determine the distribution of quantitative variables and Levene's test was used to evaluate the variance homogeneity of the variables. The quantitative variables with normal distribution were analyzed by means of the Student's T test. p values <0.05 were considered significant.

This study was approved by the Ethics Committee of the Faculty of Medicine of the Universidad Nacional Mayor de San Marcos, act 1812 with project code 0013.

KEY MESSAGES

Motivation for the study: Older adults have an immunosenescent immune system, so it is convenient to identify virulence factors in the bacteria that can alter immune response.

Main findings: The *Escherichia coli* carrier of the *pap GII* gene induce greater tissue damage, which increases the concentration of iron and red blood cells. Besides, there is a generalized presence of *nanA* gene, which is important for older adults during sepsis stages.

Implications: The findings contribute to the study of the immunological response in older adults with UTI, and it is the first report in Peru about the frequency of virulence factors in uropathogenic *Escherichia coli* in gerontological nursing homes.

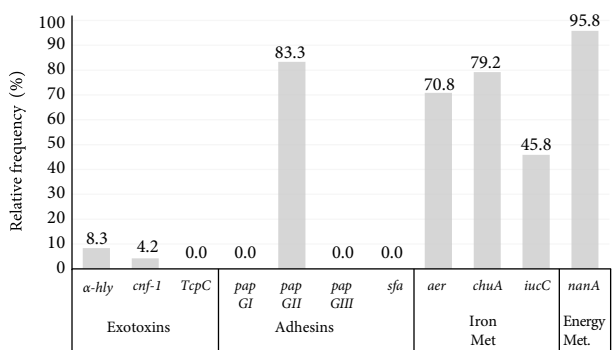
FINDINGS

Twenty-four urine samples from older adults with UTIs by *E. coli*, from gerontological nursing homes, were analyzed. The most frequent virulence genes were *nanA, pap GII, aer, chuA* and *iucC*. Genes α-*hly* and *cnf-1* were found in low proportion and the following genes were not found: *TcpC, pap GI, pap GIII* and *sfa* (Figure 1).

Regarding the immune and biochemical response of older adults to the various virulence genes evaluated, a concentration of 37.6 red blood cells/μL and 193.4 μg/L of iron was observed in patients carrying the *pap GII* gene, values were significantly higher in patients infected with *E. coli*, but without this gene. In addition, patients with strains carrying the *pap GII* gene showed a positive tendency towards having a higher urine antioxidant capacity regarding the ABTS•+ test (1348.7 vs. 634.8 Eq-real Vit C μg/mL) (Table 1). On the other hand, a higher concentration of leukocytes (p = 0.070) was observed in patients infected with *E. coli* carriers of the three genes related to iron metabolism evaluated *(iucC, aer and chuA)* (Table 2).

DISCUSSION

This study evaluated the immune and biochemical response in the urine of hospitalized older patients infected by UPEC (the main etiological agent of UTIs). One of the main findings was that the presence of the *pap GII* gene induced he-



Met: Metabolism

Figure 1. Relative frequency of virulence genes in uropathogenic Escherichia coli in older adults from nursing homes.

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Tabla 1. Immunological and biochemical markers compared to the presence of the *pap GII* gene.

Immunological and biochemical markers	Presence of *pap GII* a	Absence of *pap GII* [a]	p value [b]
Leucocytes/µL	270.1 ± 240.7	225.8 ± 126.0	0.729
Red blood cells/µL	37.6 ± 37.4	12 ± 8.2	0.010
Iron (µg/L)	193.4 ± 139.6	85.3 ± 26.2	0.004
IL-1β (pg/mL)	375 ± 293.2	147 ± 98.8	0.144
TNF- α (pg/mL)	65.2 ± 35.0	94.5 ± 61.5	0.195
ABTS Eq- real Vit C (µg/mL)	1348.7 ± 1455.7	634.8 ± 299.0	0.059
FRAP (mM)	1.006 ± 0.416	0.801 ± 0.332	0.366

[a] Mean ± standard deviation; [b] Student's T-test.
FRAP: Fluorescence recovery after photobleaching; ABTS: 2,2'-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid.

maturia and a higher iron concentration in the urine. The frequency of virulence genes important in the pathogenicity process of UTIs was also evaluated. Another important finding was that 95.8% of UPEC presented the *nanA* gene.

N-acetylneuraminic acid, an important substrate in the energetic production of *E. coli*, is split by the N-acetylneuraminic lyase (nanA) enzyme into pyruvate and N-acetyl-D-manosamine [8]. The presence of nanA enzyme generates high competitiveness in the bacteremia-producing UPEC; and although its function in UTI pathogenesis in murine models is less relevant [8], results indicate the existence of a high risk of developing sepsis in older adults with bacteremia.

On the other hand, exotoxins have a role in the pathogenesis of UTIs. Neutrophils, the main line of defense in UTIs, are lysed by high doses of α-hemolysin (HlyA) and, in addition, bladder cells in low doses are exfoliated [9], which deteriorates the body's two main lines of defense [3]. Nevertheless, we obtained a frequency of 8.3% in the studied UPEC, which is lower than what was found in other studies carried out in geriatric population [10,11]. Likewise, the exotoxins, such as the necrotizing cytotoxic factor type 1 (CNF1) genetically related to HlyA, induce a rearrangement of the neutrophilic cytoskeleton through the activation of the Rho GTPase type enzymes [9]. In accordance with what was reported by *α-hly*, we found a UPEC carrier of the *cnf*-1 gene.

The TcpC protein recently described as interfering in the production of proinflammatory cytokines in the UTI [12] was not found in any of the evaluated UPEC, which may reflect the absence of the type IV pathogenicity island, dependent on its horizontal transfer, or the recent evolutionary acquisition of the gene in extra-intestinal *E. coli* [12].

Regarding the immunological and biochemical response observed in the older adults, iron is scarce in the urinary fluid and is indispensable for bacterial metabolism [13,14], the microorganisms that infect the urinary tract must have the capacity to capture and compete for iron assimilation [14]. UPECs have three systems of iron uptake (siderophores, hemophores and direct iron uptake in its ferrous state), which are reported to have increased expression *in vivo* [15]. On the other hand, iron restriction is an immune defense mechanism used by the host to limit bacterial proliferation [16]. Although we could not find significant differences between immunological and biochemical markers and genes associated with iron metabolism, it can be observed that patients with UPEC, carriers of the *iucC, aer* and *chuA* genes, tend to have higher leukocyte concentrations,

Table 2. Immunological and biochemical markers compared with the presence of *iucC+aer+chuA* genes.

Immunological and biochemical markers	Presence of *iucC+aer+chuA* [a]	Absence of *iucC+aer+chuA* [a]	p value [b]
Leucocitos/uL	391.7 ± 226.6	209.6 ± 206.5	0.070
Hematíes/uL	35.3 ± 36	32.6 ± 36.4	0.903
Hierro (ug/L)	210.6 ± 140.2	160.9 ± 132.6	0.418
IL-1β (pg/mL)	421.1 ± 280.5	302.4 ± 284.4	0.359
TNF - α (pg/mL)	82.8 ± 24.2	64.9 ± 45	0.332
ABTS Eq-Vit C real (ug/mL)	1763.7 ± 2349.6	1009.9 ± 626.8	0.435
FRAP (mM)	0.957 ± 0.307	0.978 ± 0.447	0.911

[a] Mean ± standard deviation; [b] Student's T-test.
FRAP: Fluorescence recovery after photobleaching, ABTS: 2,2'-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid.

which may indicate a greater immune stress. Besides, the presence of *chuA* gene and other siderophore systems are highly concentrated in strains that cause recurrent UTI [17], a common situation in gerontological resting centers.

Finally, the *pap GII* gene is a virulence factor whose function is to ensure the adhesion of UPEC to renal tissue. The association of *pap GII* gene with pyelonephritis stages has been documented [6], besides, it has been proposed that it confers competitive advantage during bacteremia stages by UTI [8]. Some evidence indicates that its presence is not determinant in stages of pyelonephritis, therefore, its function is not yet conclusive in the process of infection in the urinary tract [18].

Results obtained show that patients with UPEC positive for the *pap GII* gene had higher iron concentration compared to patients with UPEC negative for the *pap GII* gene. It has been found that the interaction of *pap GII* with its receptor induces rapid transcription of the airS gene in UPEC in urine, which has a fundamental role in the activation of the siderophore systems (aerobactin/enterobactin) [19] by decreasing the concentration of iron in urine. In the results obtained we did not find this association. However, this could be explained by the higher concentration of red blood cells and hemolysis in this group of patients. The adhesion of the *pap GII* protein activates the synthesis of cytokines [20], causing the rupture of blood vessels located in the lamina itself. On the other hand, the ABTS•+ test describes that in patients with positive *pap GII* UPEC there is a tendency to have greater antioxidant capacity, which could be due to higher iron concentration, which interferes with the assay, although, this tendency could also be explained by the deficiency of neutrophils to produce reactive oxygen substances. Recent studies have described an increase in the neutrophil subpopulation CD16/CD62L in the OAP, which presents a lower level of response to cytokine stimuli [4].

This study has several limitations. First, it was not possible to record information from the patients regarding their clinical condition, infection stage, use of antibiotics, infection recurrence. Second, the low number of urine samples analyzed, which increases the probability of obtaining a type II error. Third, we do not complement our studies with gene expression analysis, so we do not know if the genes evaluated are indeed expressed by the bacteria.

In conclusion, *E. coli* carrying the *pap GII* gene can induce greater tissue damage that possibly favors a higher iron concentration in the urine, which stimulates its increase. In addition, patients infected with *E. coli* with *pap GII* show a positive tendency to have greater antioxidant capacity, which may be due to the deficiency of neutrophils recruited in the OAP during the production of ROS. Finally, the generalized presence of *nanA* gene is important due to its high relevance in sepsis stages.

Authors' contributions: AGR, HJBP, YLLM and DVRC conceived of the article. AGR, CG, SFIV, PW, HJBP and YLLM collected the data and did the statistical analysis. AGR, PW, HJBP and SSC wrote and approved the final version.

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