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ARTIGO ORIGINAL

O INCREMENTO NO DIAGNÓSTICO DE INFECÇÕES DO TRATO URINÁRIO PELA INCLUSÃO DA COLORAÇÃO DE GRAM NA URINÁLISE

THE IMPROVEMENT OF URINARY TRACT INFECTIONS DIAGNOSIS BY THE INCLUSION OF GRAM STAINING IN URINALYSIS

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RESUMO

O exame de urina é teste auxiliar para o diagnóstico de infecções do trato urinário (ITU). Esse apresenta baixa sensibilidade e especificidade na detecção de ITU e bacteriúria assintomática (BA). O estudo busca reforçar a relevância dos resultados da análise de urina com coloração de Gram nas lâminas de sedimentos de urina. Foram estudadas 235 amostras de urina coletadas em ambulatório da Faculdade de Medicina do ABC. Os resultados indicam maior positividade de ITU em exames de urina, coloração de Gram e urocultura (72,7%) no sexo masculino. Entre as amostras negativas para a cultura de urina, 11 foram positivas usando a coloração de Gram, sugerindo que esse método pode detectar ITU por bactérias exigentes ou BA. A concordância entre a coloração de Gram na urina e a cultura de urina foi de 94,98% e substancial (Kappa = 0,777), em oposição a 81,59% entre as análises de urina e cultura de urina e moderada (Kappa = 0,448). A análise de urina mostrou sensibilidade de 89,66% e especificidade de 80,48%, mas a coloração de Gram na urina mostrou 86,21% de sensibilidade e especificidade de 96,94%. A inclusão da coloração de Gram no exame de urina melhora a especificidade, aumenta a detecção de bactérias exigentes e casos de BA, levando a uma diminuição no número de falsos positivos e auxiliar na definição da antibioticoterapia sem a cultura de urina. Os resultados sugerem que a inclusão da coloração de Gram no exame de urina melhora o diagnóstico de ITU, sendo relevante em procedimentos hospitalares.

Descritores: Exame de urina; Cultura de urina; Infecção do trato urinário; Coloração de Gram; Sedimento urinário.

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ABSTRACT
Urinalysis is an auxiliary test for diagnosing urinary tract infections (UTI). However, it is a test that has low sensitivity and specificity in detecting UTI and asymptomatic bacteriuria. This study seeks to strengthen the relevance of urinalysis results, through the use of Gram staining on urine sediment slides. Urinalysis, urine Gram staining and urine culture tests were carried out on 235 urine samples collected in an outpatient department. During the period of this outpatient study, there was a higher incidence of requests for urinalyses, urine Gram staining and positive tests for UTI (72.7%) for males. Among the negative samples for urine culture, 11 were positive using Gram staining, suggesting that this method may detect UTI by fastidious bacteria, or cases of asymptomatic bacteriuria. The agreement between urine Gram staining and urine culture was 94.98% and substantial (Kappa=0.777), as opposed to 81.59% between urinalyses and urine culture and moderate (Kappa=0.448). The urinalysis method showed sensitivity of 89.66% and specificity of 80.48%, but Gram staining in urine showed 86.21% sensitivity and specificity, reaching 96.94%. The inclusion of Gram stain in urinalysis improves the specificity, increase the detection of fastidious bacteria and suggestive cases of asymptomatic bacteriuria (AB) probably leading to a decrease in the number of false positives and could assist in defining antibiotic therapy without urine culture. Due to these data, the inclusion of Gram stain in urinalysis improves the diagnosis of UTI, being relevant mainly in hospital procedures.

Keywords: Urinalysis; urine culture; urinary tract infection; Gram staining; urine sediment.

1. INTRODUCTION

Around 150 million people are diagnosed with UTI every year in Brazil. Among bacterial infections, it is the most frequent one, accounting for 80 positive results out of every 1,000 tests performed[1]. These infections are mainly caused by enterobacteria, with *Escherichia coli* responsible for 80% to 90% of the cases[2][3][4]. Other enterobacteria, gram-negatives and gram-positives are also found, as well as opportunistic and protozoan fungi[2][3]. The frequency of antibiotic resistant bacteria causing UTI has increased[3]. Studies show that the population most affected by UTI is women, pregnant women, menopausal women and elderly people[5][6][7][8]. Tests done in outpatient departments are not always accompanied by the characteristic symptoms of UTI, such as dysuria and urinary frequency and urgency. In such cases, urinalysis is often supplemented with urine culture[9][10]. In emergency rooms, patients normally report typical symptoms of UTI, and urinalysis is the test of choice for making a diagnosis, due to its quickness[11][9][10]. The examination of urine in urinalyses consists of a physical test, chemical test and microscopic examination of urine sediment. This test is mainly used for diagnosing urinary tract infections in outpatient departments of emergency or auxiliary hospitals[12][9].

Women are more prone to UTI, since they have open urinal, vaginal and rectal cavities in intimate contact, which facilitates contamination and infection[13][14]. Around 3.5% of the female population can have bacteria and leukocyturia present in their urine without any clinical symptoms, referred to as AB[14]. AB is defined by the presence of $> 10^4$ (colony forming unit) CFU/ml of bacteria in urine, pyuria and the absence of clinical symptoms[7]. Bacteriuria is more frequent among women, pregnant women and elderly people, which is a concern to physicians, since AB patients can develop

complications in the urinary system, such as cystitis, pyelonephritis and prostatitis[7][8][14]. This type of progression is more common among pregnant women and studies have shown that antibiotic therapy, in cases of AB or UTI, prevents complications[15]. The use of antibiotics for treating AB in other patients is debatable, since a comparison of treated and untreated patients does not indicate that doing so made a change in their clinical situations[16]. It has been observed that bacteria from the normal flora of the distal urethra, vagina and intestinal tract can cause AB. This bacteriuria in the urinary tract can prevent colonization by uropathogenic bacteria and the development of UTIs[17][7]. Apart from the bacteria from normal flora, AB and UTI can be caused by fastidious bacteria, which do not grow in standard urine culture tests[9]. Dune and collaborators demonstrated that *Lactobacillus*, *Streptoccoccus* and *Gardnerella* were detected through the expanded-spectrum enhanced quantitative urine culture protocol as causes of UTI, and that these bacteria are not cultured using the standard urine culture protocol[9].

In caring for elderly patients, pregnant women and menopausal women, urinalysis is used in outpatient departments and emergency rooms to investigate possible UTI or AB. The specificity and sensitivity of this test are crucial for diagnosis[16][9][11]. In urinalysis, the evaluation of leukocyte esterase, pyuria and the presence of nitrite may indicate the presence of bacteria in urine and possible infection[18][19]. Many clinicians interpret such positive results as indicators of infection and use them to map out an antibiotic therapy[14]. The correlation between urinalysis and clinical data and absence of urine culture can lead to improper use of antibiotics and increased bacterial resistance[16][10]. This correlation has been studied and the results indicate low sensitivity of esterase and nitrate tests with positivity for the presence of bacteria in urine[20]. Other authors have obtained results that emphasize the importance of urine sediment and the quantification of leukocytes and bacteria, for greater specificity in the detection of bacteria in urinalysis[21][22][23]. The Infectious Diseases Society of America recommends urine culture for confirming the diagnosis of UTI or AB[7][16].

The current literature indicates that urinalysis has low sensitivity and specificity to detect UTI and AB, and its predictive capacity improves by adding the quantification of leukocytes and bacteria in the urine sediment[9]. Given these data, this study proposes to examine the relevance of including Gram stain in urine sediment in urinalysis, in order to improve the test's predictive ability to diagnose UTI and AB.

2. METHODOLOGY

2.1 Urine sample

The study was conducted in September 2015 with outpatients served by the Clinical Laboratory of the ABC School of Medicine (FMABC). During the study, 3,904 patients received care in the Specialties Outpatient Department of Praia Grande, the Specialties Outpatient Department of Mauá, the Specialties

Outpatient Department of Santo André and the Women's Hospital – Maria José dos Santos Stein in Santo André, all in the state of São Paulo, Brazil. A total of 989 patients had requests for urinalysis. The inclusion criteria were patients with requests for both urinalysis and urine culture and being more than 20 years of age. The exclusion criteria were surgical inpatients, pregnant women and those using antibiotics for less than 30 days. The patients were approached while waiting to do lab tests, the study was explained to them and consent was given by signing a free and informed consent form. This study was approved by the Ethics Committee (CAAE: 67420417500005510). Of the 939 patients with requests for urinalysis, 235 were included in the study and 754 were rejected. Of these 754, 723 only had requests for urinalysis, 12 were pregnant women, 15 were less than 20 years old and 4 had been using antibiotics for less than 30 days.

The urine samples of the patients who agreed to participate in the study were divided into three parts and sent to the clinical analysis laboratory of the ABC School of Medicine for the urinalysis and to the microbiology laboratory of the ABC School of Medicine for the urine culture and to prepare the urine sediment slide and Gram staining. The results were compared after performing the tests on the 235 samples.

2.2 Urinalysis

The samples sent to the clinical analysis laboratory of the ABC School of Medicine were examined through urinalysis. This test includes a physical and chemical analysis and an examination of the sediment of the urine samples[2].

2.3 Urine culture

The samples sent to the microbiology laboratory of the ABC School of Medicine underwent the standard urine culture protocol[24][9][10].

2.4 Urine sediment slides and Gram staining

For the preparation of the slides with urine sediment, 10 ml of urine was centrifuged in 15 ml conical tubes for 10 minutes at 2,500 RPM. The supernatant was discarded, and the pellet was resuspended in 200 µl of sterile saline solution. This material was loaded on a glass slide and fire-dried for later Gram staining[23][25]. The sediment was analyzed with a standard optical microscope at 100x magnification. Ten fields of the slide were examined, and pyuria was defined as more than five leukocytes per field of the slide[26]. The number of bacteria found in the sediment was classified as absent, rare, moderate and numerous; a count of 20 fields of the slide at 100x magnification was standardized. The mean presence of bacteria on the slides was classified as: rare (1 to 9 bacteria),

moderate (10 to 20 bacteria) or numerous (> 20 bacteria)[27][25]. Urine with absence or rare presence of bacteria was considered negative for this method, whereas urine with the presence of numerous bacteria was considered positive for UTI. It was considered that samples with a moderate number of bacteria using the urine Gram stain technique were interpreted as bacteriuria. The presence of other microorganisms, such as yeast and protozoa, was also considered relevant.

2.5 Statistical analysis

The association between the biochemical characteristics and analysis methods was tested using the chi-square test. To analyze agreement with urine Gram staining, urine culture and urinalysis, a Kappa statistical analysis was performed. Values for sensitivity, specificity and positive (LR+) and negative (LR-) likelihood ratios were calculated, with the urine culture method as the gold standard. The level of significance was 5%[28].

3. RESULTS

During the period of the study, 3,904 tests were requested from the laboratory of the ABC School of Medicine, of which 989 were requests for urinalysis (47.2% for men and 52.8% for women). For this study, only patients with requests for urinalysis and urine culture were included, totaling 235 patients. Elderly people represented 59.1% of the sample, 66.1% of whom were men and 33.9% women. Of the 235 urine cultures, 29 samples were positive for UTI: 54.5% were elderly people, 72.7% men and 27.3% women. Of the 206 samples that tested negative for UTI with urine culture, 20 had a moderate number of bacteria (Table 1). Of these 20 samples, 9 had growth of multiple microorganisms and were excluded from the study; 11 had growth of only one microorganism and were considered positive bacteriuria. In relation to Gram staining, of the 11 samples with moderate amounts of bacteria, 7 were Gram-positive and 4 were Gram-negative. Of these samples, 27.3% also had leukocyturia: 63.6% elderly people, 54.6% men and 45.4% women (Table 2). Of the 235 samples that participated in the study with urinalysis and urine culture, 12% of this total number of samples were positive for UTI. Around 5.3% of the 235 samples tested positive for bacteriuria using the Gram stain technique (Table 2), enabling increased capacity to detect bacteria through urinalyses and leading to increased detection of bacteria in urine by including Gram staining of urine sediment.

Comparing the results of the three different techniques, it was noted that bacteria detection capability is higher using the urinalysis method (28.03%; $p<0.001$), in relation to the rates obtained by urine Gram stain (13.81%) and urine culture (12.08%) (Table 3). As for the type of bacteria detected, there was a higher proportion (71.43%) of Gram-negative bacteria using the urine culture method compared to urine Gram stain (66.6%). In this study, a higher proportion of Gram-positive bacteria by

Gram staining (33.3%) was detected, compared to the urine culture method (25%), albeit not significant (p=0.342). These results indicate that Gram staining can be used to improve the diagnosis of UTI, caused mainly by Gram-positive bacteria (2)(3). The analysis of the presence of leukocytes showed a higher proportion (p<0.001) of samples with leukocytosis, using the urinalysis method (26.36%) as opposed to the urine Gram stain method (11.3%). However, the absence of leukocytosis has been more evident through the Gram stain method (88.51%) (Table 3). Lack of pyuria cannot be a determinant for ruling out UTI, since 21% of these infections occur without the presence of leukocytes[26].

The urine culture tests identified 11 different species of bacteria and the yeast-form fungus *Candida tropicalis* (Table 4). The bacteria detected the most often were *Escherichia coli*, followed by *Klebsiela pneumonia* and non-saprophyticus coagulase-negative *Staphylococcus* (Table 4). Other species were also found in the patients examined, but at a lower frequency (Table 4).

The agreement between urine culture and the results obtained from urine Gram stain and urinalysis was 94.98% (p<0.001) and 81.59% (p<0.001), respectively (Table 5). The agreement between urine Gram stain and urine culture was substantial (Kappa=0.777), and moderate between urine culture and urinalysis (Kappa=0.448) (19) (Table 5). In addition, in relation to the urine culture method, the Gram-stained urine sediment method had higher values of specificity (96.19%) and accuracy (94.98%) than urinalysis which had 80.48% specificity and 81.59% accuracy (Table 5).

4. DISCUSSION

This study investigates the relevance of Gram staining of urinary sediment in urinalysis and whether this diagnostic approach might improve the predictive value of Gram staining for the diagnosis of UTI, AB and antibiotic therapy indication. If so, it could enable improving the diagnosis of UTI through combining urine Gram staining with urinalysis. Although urinalysis is the most frequently used method in clinical practice, quantification of the bacteria and leukocytes present is presumptive. Only when there are numerous bacteria and leukocytes can UTI be conjectured. This study showed that urinalysis is sensitive for detecting bacteria (89.66%), but less specific for predicting when this presence indicates UTI (80.48%). In turn, urine Gram staining proved to be a less sensitive (86.21%) but more specific (96.19%) method for detecting bacteria in urine. In the literature, urine culture is deemed to be the gold standard for diagnosing UTI. It has also been considered a standard for assessing the performance of urine Gram staining and urinalysis for predicting UTI. When comparing the UTI predictive capability of urinalysis and urine Gram staining with urine culture, it was noted that there is a moderate relationship between urinalysis and urine culture (Kappa=0.448) and a substantial one between urine Gram staining and urine culture (Kappa=0.77). These findings coincide with the study by Yodoshi and collaborators 2019, which reported substantial agreement between urine Gram staining and

urine culture (Kappa=0.784). It can be argued that the use of urine Gram staining substantially increases the ability to detect and classify by morphology and staining characteristics the bacteria present. This additional information can facilitate the diagnosis of UTI and guide treatment[26].

The possibility of distinguishing UTI from AB, particularly in patients with nonspecific symptoms, is still a challenge[29]. However, this study fills a gap in the literature regarding the value of the basic approaches for discriminating between acute and sub-acute UTI. In this sense, the study is highly relevant, since this differentiation will be reflected in better decisions on antibiotic treatment or not, thereby preventing the increased prevalence of antimicrobial resistance by bacteria from the urinary tract[30]. In this study, 11 samples with moderate presence of bacteria in Gram-stained urine sediment, 7 were classified as Gram-positive and three as Gram-negative. The fastidious bacteria that most often cause UTI or AB are *Lactobacillus*, *Streptococcus* and *Gardnerella*, all Gram-positive[9]. Therefore, urine Gram staining may indicate the presence of fastidious bacteria, enabling changes to the standard urine culture protocol, in order to confirm infection and avoid future complications[8][14]. Lack of definition in relation to many key issues complicates the approach to the spectrum of syndromes included within the broad category of UTI. These issues include poorly defined clinical criteria for diagnosing UTI; limited instruction on the use and interpretation of diagnostic tests; difficulty distinguishing AB from UTI; and challenges in the selection of empirical antimicrobial therapy, among others[30]. Therefore, the findings of this study of increased detection of bacteria through Gram staining may be decisive for physicians to make more accurate decisions about UTI treatment, since in clinical outpatient consultations, physicians normally only request a urinalysis for investigating UTI[11]. Most antibiotics that have been incorrectly prescribed for UTI are due to diagnoses based on nonspecific findings, such as leukocytosis, presence of bacteria, weakness and discomfort[31]. The risk of harm in delaying UTI treatment in clinically stable patients is low. In general, the risk-benefit ratio favors a more cautious approach to diagnosing and prescribing antibiotics for UTI when signs and symptoms are not present[32]. The present study showed that a Gram staining analysis, where the morphological and staining characteristics of the bacteria present are observed, enabled a better qualitative assessment of the elements present in the urine sediment, as well as quantification of the leukocytes, resulting in increased detection of cases suggesting AB or infection from fastidious bacteria. Combining this technique with urinalysis would permit better assessment of possible UTI in patients and more effective tailored treatment[26]. Therefore, greater specificity in determining the morphological and staining characteristics of bacteria using the Gram staining method can increase UTI diagnosis in hospital routines. Similarly, Yodoshi and collaborators showed that Gram stain-based antibiotic therapy results in a better selection of antibiotics compared to empirical therapy[26].

Unfortunately, this study did not assess the typical symptoms of UTI in the volunteers, but it is possible to suggest that increased use of Gram staining in urinalysis could indicate possible AB. In clinical outpatient consultations, physicians preferably request a urinalysis for investigating UTI[11]. In view of this, the inclusion of urine Gram staining in urinalysis would truly enhance sensitivity and specificity in the detection of bacteria and leukocytes. Outpatients with AB and UTI caused by fastidious bacteria could be referred for subsequent confirmation of the diagnosis. The progression of microbiological contamination of AB patients could be monitored without the need for frequent urine cultures.

The literature shows that women are more prone to urinary infections than men due to their genitourinary system[14]. In the analysis of 989 requests for urinalysis during the period of the study, 47.2% were for men and 52.8% for women. There was no trend of outpatient clinical staff requesting more urinalyses for women than men. Of the 235 samples included in the study, 66.1% were from men and 33.9% from women. Among the 29 positive cases, 72.7% were men and 27.3% women. The results concurred with a study by Yodoshi and collaborators, in which he examined cases of UTI in children up to 36 months of age. The study found that 55% of the requests for urinalysis and urine cultures were for male children and that 66% of the positive cases in the urine culture also corresponded to the same sex. The findings of the present study and those of Yodoshi contradict the literature that was examined[26]. Both studies address the frequency of UTI in women. Foxman and collaborators, argued that 3.5% of women may have AB. Considering that the female anatomy is conducive to bacterial contamination in the genitourinary tract, it can be supposed that the percentage of AB in the female population would be higher than indicated. This hypothesis is relevant in view of the fact that 5.3% of the population examined in the study had positive bacteriuria and negative urine culture. Consequently, these outpatients are not being identified for evaluation and treatment, which could lead to future complications, such as cystitis and pyelonephritis[8][14]. A crucial point in this and Yodoshi's study is the lack of relationship between UTI and symptoms, since this data was not examined in the volunteers, and the symptomatic information was vague in children up to 36 months of age[26]. Without this information, cases of AB are inconclusive. It can also be suggested that, in outpatient terms, men have more symptomatic complaints of UTI than women, leading to a higher frequency of test requests for men. Women who go to outpatient facilities without symptoms are not examined or diagnosed with UTI or AB. A slightly larger number of elderly people with requests for urinary tests were noted in this study, but the differences were considered irrelevant. Further light could be shed on these hypotheses and questions, in another study with a longer time frame and assessment of typical UTI symptoms.

The test results showed the presence of 12 different types of bacteria. The most detected bacterium was Escherichia coli, which coincides with the clinical data found in the literature[3][26]. This

indicates that the genitourinary system is conducive to urinary infection by bacteria from the intestinal microbiota[2][3][4].

5. CONCLUSION

Based on these findings, it can be concluded that the inclusion of Gram stain in urine sediment in urinalysis makes the test more specific to detect UTI, AB and infections caused by fastidious bacteria. Gram stain allows bacteria to differentiate and target antibiotic therapy, as well as to evaluate the inflammatory process due to the typing and quantification of white blood cells. The results allow us to suggest the inclusion of Gram stain in urinalysis as a way to improve the predictive capacity of the test, reducing false positives and avoiding the generation of antibiotic resistant bacteria. The higher frequency of urinary tests in men than women, as well as the positive results in urine cultures, deserves attention and further studies.

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TABELAS

Tabela 1. Indication of age and sex of outpatients who used the Clinical Analytical Laboratory service of FMABC in September 2015.

	Tests requested	Urinalysis	Urinalysis + Urine culture	Urine culture (+)	Urine culture (-) Gram staining moderate
Total No. of volunteers	3,904	989	235	29	11 (5.3%)
Elderly people (%)	-	-	59.1	54.5	63.6
Male (%)	-	47.2	66.1	72.7	54.6
Female (%)	-	52.8	33.9	27.3	45.4

Tabela 2. Quantification and classification of the bacteria present in Gram- stained urine sediment.

Bacteria	Total (+)		Gram (+)	Gram (-)
Numerous	33		11	22
Moderate	20	11	7	4
		9	Mixed culture of microorganisms	

Tabela 3. Agreement, sensitivity, specificity, accuracy and positive and negative likelihood ratios of Gram staining and urinalysis when compared with urine culture (gold standard).

Parameters	Gram staining	Urinalysis
Agreement (%)	94.98	81.59
Kappa	0.777 (CI: 0.675-0.898)	0.448 (CI: 0.321-0.575)
p-value*	<0.001	<0.001
Sensitivity (%)	86.21	89.66
Specificity (%)	96.19	80.48
Accuracy (%)	94.98	81.59
LR+	22.62	4.59
LR-	0.14	0.12

*Kappa

CI: Confidence interval 95%

Tabela 4. Identification of bacteria isolated in urine culture.

Microorganism	Number of samples
Escherichia coli	15
Klebsiella pneumonia	3
Non-saprophyticus coagulase-negative staphylococcus	2
Staphylococcus aureus	1
Staphylococcus saprophyticus	1
Streptococcus agalactiae	1
Enterococcus faecalis	1
Enterococcus faecium	1
Enterobacter aerogenes	1
Acinetobacter baumannii calcoaceticus	1
Candida tropicalis	1
Enterobacter cloacae	1

Tabela 5. Association between biochemical parameters and analysis methods.

Biochemical parameters	Method			p*
	Urinalysis (n=235)	Gram staining (n=235)	Urine culture (n=235)	
Presence of bacteria		n (%)		
No	168 (71.48)	202 (85.95)	206 (87.65)	<0.001
Yes	67 (28.51)	33 (14.04)	29 (12.34)	
Types of bacteria				
Gram (+)	-	11 (33.33)	9 (25.00)	
Gram (-)	-	22 (66.66)	20 (71.43)	0.342
Yeast cells	-	1	1 (3.57)	
UTI (leukocytes > 5/lpf.)				
No	172 (73.19)	208 (88.51)	-	<0.001
Yes	63 (26.80)	27 (11.48)	-	

* Chi-square
lpf - leukocyte per field