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Research Article

Cytochemical Method for Staining Active Leukocytes in Tubulo-interstitial Bacterial Nephritis - A Comparative Study

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Abstract

The demonstration of active, live leukocytes (glitter cells) in the final urine sediment is a marker for the presence of an inflammatory process in the excretory system of the kidneys in tubulointerstitial bacterial nephritis, acute pyelonephritis. These cells are best seen using a phase-contrast microscope. Unfortunately, these types of microscopes are expensive, their maintenance is complicated, and their use requires specially qualified and trained personnel. Therefore, we decided to experiment in two directions: 1. To use the capabilities of a conventional light microscope to detect active leukocytes in the urine sediment, 2. To make a comparative analysis between our proposed original, fast and inexpensive method for demonstrating active leukocytes in the urine sediment by staining with a 1% solution of the dye Methylene-Blau, on the one hand, and our modified method for staining cells in the urine sediment with Eosin-Yello (Eo-Yell) (which is standardized by WHO for demonstrating active, live spermatozoa). Our method for staining polymorphonuclear leukocytes in urine sediment with Methylene-Blau well differentiates the population of active and the population of inactive leukocytes, and the quality of cytological preparations with our method provides an excellent and suitable alternative to cytological staining in the examination of urine sediment for evidence of acute kidney infection. Our proposed method is a good urinary "biomarker" for acute kidney disease. When used properly, urine sediment results alert health care professionals to the presence of kidney disease, while providing diagnostic information to localize kidney damage, guide therapy, and aid in prognosis. Our published method can be used to demonstrate living cells (living tissues) - a current issue related to transplantation. But this is a subject of future research and trials.

Keywords

Urine Sediment, Cytodiagnosis, Viable Cells /Cultures/, Glitter Cells, Acute Pyelonephritis

1. Introduction

The demonstration of active, live leukocytes (glitter cells) in the urine sediment of the final urine is a marker for the presence of an inflammatory process in the excretory system of the kidneys in tubulo-interstitial bacterial nephritis, acute pyelonephritis. Timely diagnosis of acute pyelonephritis plays an important role in effective medical practice. In ambulatory conditions, the quick orientation in whether it is an acute inflammatory process of the kidney allows the doctor

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to discuss further lines of action with the patient already during the first visit. Therefore, the cytological examination of the urine sediment for the presence or absence of active, live, "glitter cells" leukocytes is such an important problem.

2. Material

This prospective study was conducted among fifty patients with clinically proven acute pyelonephritis who were examined for urine sediment. After permission from the ethics committee, the consent of the studied patients was obtained. A urine sediment test is an inexpensive and rapid diagnostic test. To be objective, this test must be standardized. Standardization of urine sediment includes – first morning urine, intermediate portion, which has been in the bladder for at least 4 to 6 hours, taken after showering and examined within 1 hour of voiding. The material examined is the sediment obtained after centrifugation of 10 ml urine at 400 rpm, for 5 minutes. 9.5 ml of the supernatant is removed, leaving only the precipitate, which has a higher density and collects at the bottom. The remaining 0.5 ml of sediment is homogenized and examined microscopically - a native preparation on a slide at different magnifications.

3. Method

Using the non-parametric statistical analysis that compares two paired groups - Wilcoxon test, we compared our method with the method approved by the WHO for the demonstration of active cells by Eosin-Yellou staining. The quality of staining (Methylene-Blau, Eosin-Yellou) was evaluated by considering four parameters: smear background, cell morphology, nuclear features of leukocyte cells in the sediment and overall staining (Table 1).

Table 1. *Indicators for evaluation of staining.*

	Score
*Background	
Clean	1
*Cell morphology	
Not preserved	1
Moderatery preserved	2
Well preserved	3
*Nuclear characteristics	
Dull	1
Crisp	2
*Overal staining	
Bad	1
Moderately good	2
Good	3

The maximum possible score for one indicator, considering all four parameters, was 10. The data were subjected to statistical analysis and the results were tabulated. (Table 2).

Table 2. *Comparison of staining methods for active, live, "glitter cells" leukocytes in urine sediment in patients with proven acute TIBN (our staining method with Methylene-Blau and method for active cells with Eosin-Yellow), by Wilcoxon-matched pair test.*

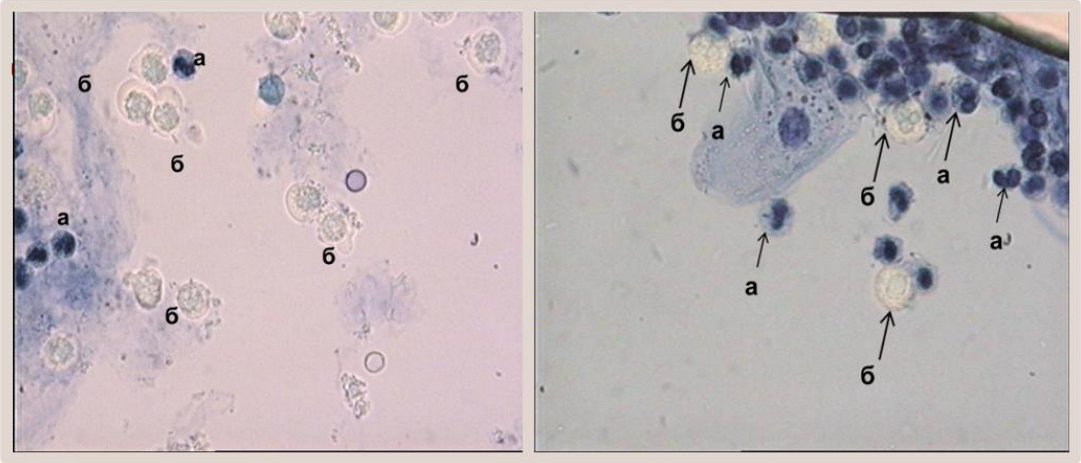
Material	Procedures	Mean	SD	Median	Mean diff	t	Z	p
Urine sediment	Methylen-Blau	2.21	0.53	2.00	-0.92	18.9	6.0354	0.00001
	Eosin-Yellou	1.28	0.19	1.00				

P< 0.05

Staining procedure: Our proposed method for staining urine sediment with Methylene-Blau is a simple, affordable and fast method, an alternative to phase-contrast microscopy, which reliably differentiates active granulocytic leukocytes (glitter cells) in the urine sediment of patients with active TIBN. Our method of visualizing urine sediment involves supravital staining with 1% aqueous Methylene-Blau, in which material and stain are placed in equal amounts, mixed and left in a humid chamber for 3-5 min, after which one drop is placed on a glass slide and pressed with a coverslip. Viewed at various magnifications (recommended also at 10x100). This method does not require the use of a phase contrast microscope (Figure 3).

Results: The quality of cells in urine sediment stained by our method with Methylene-Blau (Figure 1) was better in comparisons with Eosin-Yellou staining (Figure 2) and was statistically significant by Wilcoxon-matched pair test with P< 0.05 (Table 2). Methylene-Blau-stained urine sediment preparations showed a clear background, well-preserved cellular morphology with polymorphonuclear features, and

overall, a good staining pattern.



(a) (b)

Figure 1. *(a, b) Urine sediment stained with 1% aqueous solution of Methylene-Blau / author's method -x100/.*

2 types of leukocytes are found - one (a) has the usual size and shape, a barely distinguishable nucleus and coarse granularity in the protoplasm. The leukocytes of the second group (b) are called Sternheimer-Malbin cells. The fact that in patients with active tubulo-interstitial bacterial nephritis these (b) cells are almost always detected suggests that they could be added to the laboratory constellation for the diagnosis of active pyelonephritis. The leukocytes of the second group (b) are of increased size (1.5-2 times larger than normal), with a round shape, sometimes with vacuolization of the protoplasm. The nucleus of these leukocytes is polysegmented, divided into 2 to 4 spherical, interconnected parts, and is darker in color than their protoplasm. Granulations are observed in their cytoplasm, which are in a state of continuous chaotic (Brownian) motion.

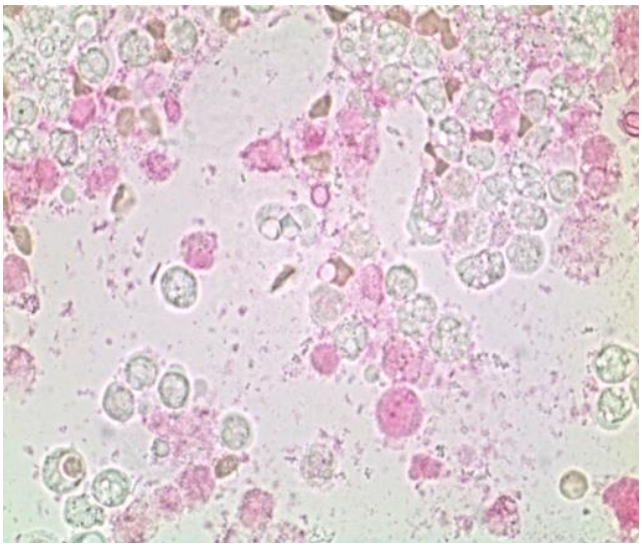


Figure 2. *Urine sediment stained with Eosin-Yellou (Eo-Y)- x100.*

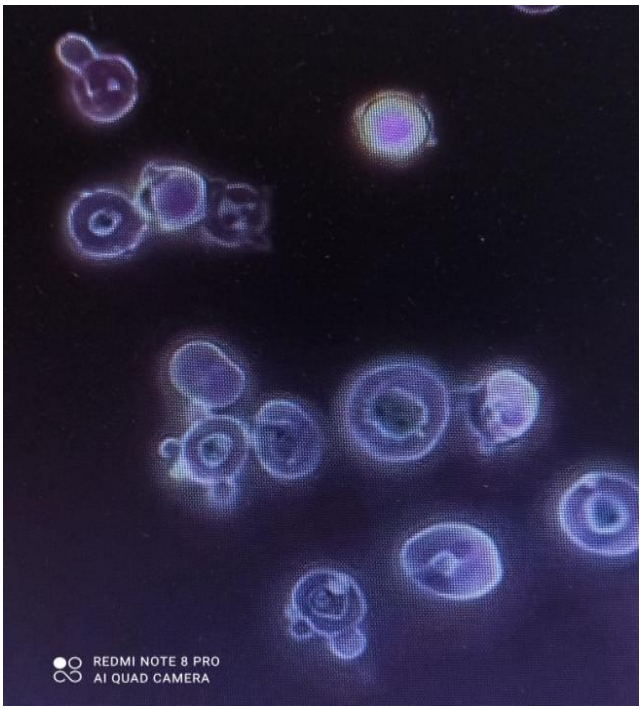


Figure 3. *Urine sediment - phase contrast microscope.*

4. Discussion

Urine sediment examination is a rapid and inexpensive diagnostic test. The detection of active representatives of the leukocyte population in the urine sediment is a condition that confirms the activity of the infectious process in the excretory system [3, 7, 10, 14]. Our method of demonstrating such active leukocyte representatives is supravital staining with a 1% aqueous solution of methylene blue (methylthioninium chloride). The protocol is as follows:

equal amounts of the precipitate and the staining solution should be mixed together and left in a humid chamber. After 3 to 5 minutes of standing time, a drop of the mixture was placed on a microscope slide and sealed with a glass coverslip. Observed at a different magnification - preferably x40; x100 (Figure 1).

For the second method, we used the WHO-adopted protocol for Eo-Y staining of viable spermatozoa. We modified the protocol by applying our technique, re-targeting it for cytological examination of urine sediment (Figure 2).

To observe the stained urine sediment [1, 5, 8] from the examined patients, we used a Carl Zeiss microscope with 10x 40 and 10x 100 magnifications, as well as unstained slides viewed under a phase-contrast microscope [2, 4, 13] to objectively demonstrate glitter cells in the urine sediments of the examined patients (Figure 3).

As with the phase-contrast microscope and the two other staining methods, we observed two types of leukocytes in the sediment [6, 9, 12]. One type has a paler coloration, they have brownian movement in their cytoplasm - the so-called young, live, active leukocytes (glitter cells), while the other type of granulocytes are smaller in size, darker in color and are the so-called old or dead leukocytes.

Our proposed method for staining the urine sediment with Methylene-Blau is a simple, affordable and fast alternative to phase-contrast microscopy, as it allows reliable differentiation of the two types of leukocytes in the urine sediment of patients with active pyelonephritis [11].

5. Conclusion

The method proposed by us for staining urine sediment with methylenblau is easy, accessible, fast and cheap, an alternative to phase-contrast microscopy, better than Eosin-Yellou staining and allows reliable differentiation of the two types of leukocytes in the urine sediment of patients with active pyelonephritis. It reliably differentiates active granulocytic leukocytes (glitter cells) in the urine sediment in patients with active tubulo-interstitial bacterial nephritis.

The method meets the requirements for cytological staining and can be done using reagents and a microscope available in any laboratory.

Demonstration of active leukocytes in urine sediment could be added to the laboratory constellation for the diagnosis of active tubulo-interstitial bacterial nephritis, with the benefit of rapid orientation to the presence of an acute infection of renal origin when looking at the sediment.

Through the simple, convenient, fast and affordable method for determining "glitter cells" offered by us, the diagnosis of active inflammatory processes of the excretory system can be facilitated and accelerated

Our method could also be used to represent the vitality of cells /cultures/ in general.

Abbreviations

WHO	World Health Organization
Eo-Yell; Eo-Y	Eosin-Yello
Glitter Cells	Young, Live, Active Leukocytes

Author Contributions

Stoyanka Tancheva is the sole author. The author read and approved the final manuscript.

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Conflicts of Interest

The author declares no conflicts of interest.

References

[1] Arkan Adnan Alhassan, Nada Abdullah Bamaarof, Faisal Ali Hamoudah, Wejdan Abdullah Alzahrani, Rowaa Mohammed Al-Juhani, Raghad Fahad Alotaibi, Huda Taha Halabi, Reem Ziad Alharbi, Fatima Aqeel Alzubaidi, Jawaher Mansour Alshorfa, Pyelonephritis: Diagnosis, and Management Approach, J Biochem Tech, 2020. 11(1): 135-138.

[2] Cavanaugh C, Perazzella MA., Urine Sediment Testing in the Diagnosis and Treatment of Kidney Disease: Core Curriculum 2019., Am J Kidney Dis. 2019 Feb; 73(2): 258-27.

[3] Chung VY, Tai CK, Fan CW, Tang CN. Severe acute pyelonephritis: a review of clinical outcome and risk factors for mortality. Hong Kong Med J. 2014 Aug; 20 (4): 285-89.

[4] Coleman KB. Cystitis and pyelonephritis: diagnosis, treatment and prevention. Primary care. 2019; 46 (2): 191–202.

[5] Cesar F. Hernandez-Arroyo, Vipin Varghese, Muner MB Mohammed, Juan Carlos Q Velez; Urinary sediment microscopy in acute kidney injury associated with COVID-19, kidney 360.2020, June 2; 1 (8): 819-823.

[6] David R Lane, Sukhjit S Takhar, Diagnosis and treatment of urinary tract infection and pyelonephritis, Emerg Med Clin North Am, 2011 Aug; 29(3): 539-52.

[7] Eremenko R, Barmatz S, Lumelsky N, Colodner R, Strauss M, Alkan Y. Urinary tract infection in ambulatory children and adolescents: an analysis of the risk of antimicrobial resistance. Isr Med Assoc J. 2020 Apr; 22(4): 236-240.

[8] Gavin J. Becker, Giuseppe Garigali, Giovanni B. Fogazzi, Advances in urine microscopy, Am J Kidney Dis. 2016 Jun; 67(6): 954-64.

[9] Hatice Yuksel 1, Elif Kilic, Aysun Ekinci, Osman Evliyaoglu; Comparison of fully automated urine sediment analyzers H800-FUS100 and LabUMat-UriSed with manual microscopy, J Clin Lab Anal. 2013 Jul; 27(4): 312-316.

[10] Ilaria Lippi, Verena Habermaas, Eleonora Gori, Valentina Virginia Ebani, Alessio Pierini and Veronica Marchetti, Urinary tract cytology: potential role in urinary tract infections in dogs, Veterinary Sciences Jun 2022; 9(6): 304.

[11] Marlis MB Habing 1, Judith Bodeus 2, Petra MC Kallenbach 3, Frank GH van der Klee, The diagnostic value of urine microscopy: a comparison of nephrologist- and laboratory-based interpretation, J Nephrol. 2022 Dec; 35(9): 2429-2431.

[12] Medina M, Castillo-Pino E. Introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol. 2019; 11: 1756287219832172. https://doi.org/10.1177/175628

[13] Perazzella MA; Urine sediment as a biomarker of kidney disease.; Am J Kidney Dis. 2015 Nov; 66(5): 748-55.

[14] Perazella MA, Coca SG, Hall IE, Iyanam U, Koraishy M, Parikh CR., Urine microscopy is associated with severity and worsening of acute kidney injury in hospitalized patients., Clin J Am Soc Nephrol. 2010 Mar; 5(3): 402-8.