EVALUATION OF PERMANENT STAIN USED FOR FIXED SMEARS OF SOME INTESTINAL PARASITES IN STAINING OF URINE SEDIMENT SMEAR[+]

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Abstract

Urine samples were collected from patients complaining of urinary tract disturbances. Twenty samples were selected depending on the presence of leucocytes, erythrocytes, epithelial cells, and bacterial cells, detected by direct microscopic examination of urine sediment smear. The selected urine sediments were subjected to staining technique, with two types of stains: supravital stain using Löefflerś methylene blue as a control stain and permanent stain for fixed stool smears of some intestinal parasites (PSFSIP). PSFSIP is a new permanent stain, is efficient and has simple and rapid procedure. Three types of cells were selected in this study. The staind cells appeared as follow: Neutrophil appeared with segmented violet nucleus located in granulated moderate blue cytoplasm; normal erythrocyte appeared as a pink colored disc, and dysmorphic erythrocyte appeared as a pink crescent, both were surrounded by dark violet line, Epithelial cell appeared with pale blue cytoplasm and round or oval dark bluish violet nucleus, while bacterial cells appeared with dark blue color. This stain is simpler and efficient in staining of the color and morphology of the cellular constituents of the urine sediment smears were found to be stable and clear for entire one year.

Key words

Urine sediment cells, Neutrophils, Epithelial cells, Erythrocytes, Permanent stain for fixed smear of some intestinal parasites.

المستخلص

أختيرت ٢٠عينة ادرار من المرضى المشتكين من أضطرابات الجهاز البولي على أساس وجـود الخلايـا البيض والحمر والخلايا الظهاريه وخلايا الجراثيم في رواسب العينات و ذلك بعد اجراء الفحص المجهري المباشر للمسحات الرطبه لرسابة جميع عينات الادرار. حضر نوعان من المسحات من رسابة كـل عينـة ادرار: النوع الأول المسحه المثبة ولونت بملون المسحات المثبة والدائميه لبعض الطفيليات المعويه وهـو ملون دائمي جديد ذو كفائه تلوينيه عاليه وتتميز طريقة استعماله بالسرعه والسهوله. النوع الثانى مسحه رطبه لونت بملون المثلين الأزرق اللوفلر للتحكم. ظهرت الخلايا فى المسحات المثبته والملونـه بـالملون الدائمى كما يلي: الخليه العدله ظهرت بسايتوبلازم حبيبي أزرق ونواة مفصصه بنفسجية، الخليه الظهاريـه

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وكانت بسيتوبلازم أزرق ونواة دائريه أو بيضويه بلون بنفسجى غامق مزرق، أما كريـــة الـــدم الحمـــراء السويه ظهرت على شكل قرص وردي محاط بخط بنفسجي غامق، أما كرية الدم الحمراء المشوهه كانـــت على شكل هلال وردي محاط بخط بنفسجي فاتح،اضافه"إلى خلايا الجراثيم التي ظهـــرت بـــشكل عـــصيات زرقاء اللون. كما وأن جميع هذه الخلايا أحتفظت بأشكالها والوانها على مدى سنه كامله وإن ذلك قد تبـــين من خلال المتابعه التى أجريت لها لمدة سنه كامله.

ومن خلال مقارنة نتائج تلوين الملون الدائمى مع نتائج تلوين ملون المثلـــين الأزرق اللـــوفلر للمـــسحه الرطبه يمكن أعتبار ملون المسحاة المثبة والدائميه لبعض الطفيليات المعويه ذو كفاءه عاليه فـــى تلـــوين الخلايا المذكوره وتساعد فى التحري عن أنواع الخلايا الموجوده فى رسابة الادرار.

Introduction :

Art of urinalysis was nearly back to 6.000 years, when Babylonian physician practiced uroscopy (examination of urine). Connection between testing urine and diabetes was made as early as 600 BC [1].

Urinalysis when performed probably is a highly reliable index of renal disease [2]. Microscopic analysis of urine concentrates shows mainly on the epithelial cells, white and red blood cells, in addition to several types of casts and bacterial cells. The diversity of cells that one can meet is cells found in the urine may belong to the reticuloendothelial cells (leucocyte, macrophage) or the epithelial system. Urinary cells can originate from: kidney, lower urinary tract, superficial lining or from a deeper source. In adult male cells can also originate from the prostate and urethra [3].

Identification of urine sediment cells (USC) is a difficult job. Some cells are not easy to identify even with most popular stains. The urine sediment is routinely examined by bright field microscopy under low and high power without specific characterization of cellular elements in urine. Several staining techniques were developed, stains like Wrightś stain or Henselś for eosinophils, required air drying and fixation, which altered red cell morphology. Supravital stains could overcome these problems. Initially a single stain like toluidine blue or crystal violat, was used. Though these stains were useful in identifying cell type, they do not differentially stain the nucleus and cytoplasm, which would be more effective. A combination of diaminoflourene and phloxine B was used, but the leucocytes turned blue black and differential count was diffcult [4] , Dinda has developed simple supravital stain using safranin O, which is effective in differentiating various cell types including eosinophils and various casts [4, 5]. The stain increases the overall visibility of the elements by increasing the refractive index [6, 7]. A drop of methylene blue solution (Löefflers) added to the sediment may aid in the recognition of cellular structures and bacteria.

A crystal- violet, methylene and safranine stain is used to help in identification of cellular elements. [7, 8, 9, 10, 11, 12, 13, 14]. Permanent slides can be made by using cytocentrifugation technique and staining with Papanicolaou's stain which provide an even more standardized sediment and uniform staining [1, 6]. A new permanent stain has been recently applied in staining some intestinal parasites (PSFSIP) [15]. It

has simple and rapid staining procedure , and this solution has a long shelf life, moreover it is efficient in staining parasitic cells in stool specimens, and differentiates them from artifacts.

The objective of this study is to evaluate the efficiency of this new permanent stain in staining the cellular components of the urine sediment in comparison to the supravital stain.

Materials and Methods.

Samples collection:

- Urine samples were collected from patients complaining from different urinary tract
  disturbances, attending a private laboratory in Al- Thawra city in Baghdad.
- 20 urine samples were selected for this study.
- Selection of urine sediments was depend on the appearance of epithelial cells, leucocytes, erythrocytes and bacterial cells in the direct microscopic examination of the wet mounts smears .

Materials and reagents

- The urine samples were collected in dry and clean containers.
- Universal litmus papers for rough P^H estimation of urine samples were applied in this work.
- 50 ml of Löeffler methylene blue of 1:2 dilutions was prepared as directed by [8].
- 50 ml of PSFSIP and 50 ml of diluent solution were supplied from the patent owner [15] (the patent includes preparation both staining solution and diluents solution).

Procedure

- P^H for each urine sample was roughly estimated
- 10 ml from each urine sample was centrifuged (1500 rpm) for 10 minutes.
- The clear supernatant of each sample was discarded, urine sediment which remain on bottom of the centrifuge tube was shaken gently, to homogenize the sediments.
- Direct wet mount of unstained urine sediment was prepared and examined under light microscope to select the suitable urine sediments.
- Two slides were prepared respectively from each of the selected urine sediments, one of these slides was stained with Löeffler's methylene blue directly, the second slide was stained with PSFSIP stain.

Staining procedure of supravital stain

- A drop of suspended sediment was put on a slide and mixed with one drop of 1:2 of
  Löeffler methylene blue solution, coversliped [10] .
- The wet smear was examined under light microscope within 5 minutes of slide preparation.

Staining procedure of PSFSIP

- The remaining urine sediment of each sample was resuspended in 0.5 ml of diluents solution.
- One drop of homogeneous suspension of urine sediment was mixed with one drop of PSFSIP solution, and then spread evenly to an area about 20 × 20 mm.
- The smear was left to dry in air.
- The dry smear was mounted with Canada balsam, and then examined under light microscope.
- During the period April 2004- April 2005, all permanent stained slides were checked periodically every 15 days by examining the quality of the stain under light microscope.

Results:

- 18 urine samples their P^H were acidic.
- 2 urine samples their P^H were alkaline.
- All urine samples were positive for leukocytes and erythrocytes. Leukocytes were predominantly neutrophils.
- 17 urine samples were positive for epithelial cells and 3 samples were negative for epithelial cells.
- 14 urine samples were positive for bacterial cells and 6 samples were negative.
- The leukocytes, erythrocytes and epithelial cells which stained with permanent staining solution, showed the same staining characteristics in all selected urine samples.

Description of cells stained by PSFSIP

Neutrophils

- These cells appeared with moderate blue granulated cytoplasm, and violet multi lobulated nuclear segments, may also appeared as a small, round violet nuclei. (Table 1, figures 1 and 2).
- **Erythrocytes**
  These cells appeared as homogenous pink colored discs or were crescent shape, surrounded by dark violet line. (Table 1, figures 1 and 2).
  Epithelial Cells.
- These cells appeared with pale blue cytoplasm and dark bluish violet rounded or oval nucleus. (Table 1, figure 1)
- **Bacterial Cells**
  These cells appeared with a moderate to dark blue color, (Table 1, figure 1), arrow
  indcates bacterial cells.

Description of cells stained by supravital Löeffler's methylene blue stain.

- **Neutrophils**
- These cells under oil immersion appeared with granulated moderate greenish blue cytoplasm with dark greenish blue multi lobulated nuclear

segments or may appeare as small , round discrete dark greenish blue nuclei. (Table 1, figures 3 and 4).

- **Erythrocytes**
- These cells appeared with very pale greenish blue, and homogenous discs surrounded by black line. (Table 1, figure 4).
- **Epithelial cells**
- These cells have pale greenish blue cytoplasm and moderate greenish blue round or oval nucleus. (Table 1, figures 3 and 4).
- **Bacterial cells**
- These cells appeared with dark greenish blue color. (Table 1, figure 3), arrow indicates bacterial cells.

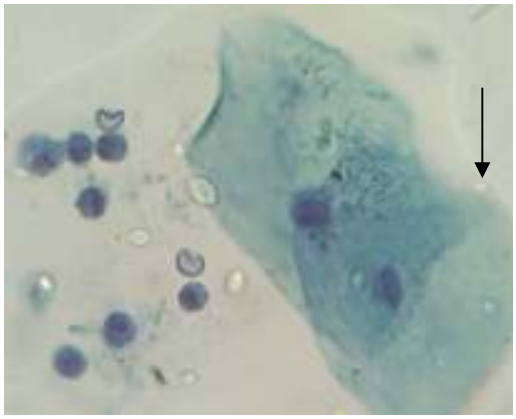

Figure (1)

Urine sediment smear stained with PSFSIP. Neutrophils, epithelial cells, dysmorphic erythrocytes, and bacterial cells are present in this smear, the arrow indicates bacterial cells adhered to epithelial cell×100.

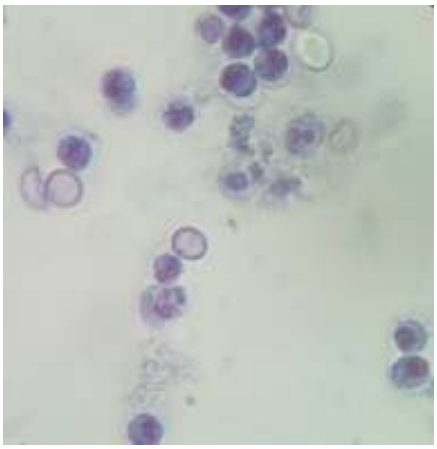

Figure (2)

Urine sediment smear stained with PSFSIP. Neutrophils, normal and dysmorphic erythrocytes are present, erythrocytes with a dark violat line surrounding the homogenous disc and the crescent. ×100.

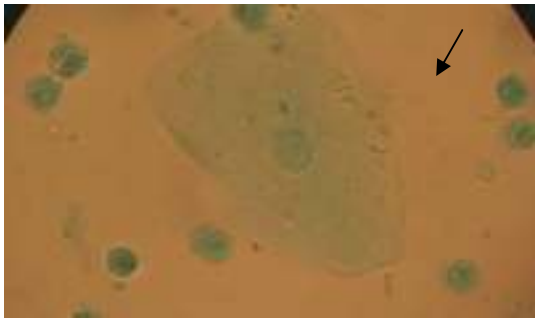

Figure (3)

Urine sediment smear stained with supravital Löeffler methylene blue stain. Neutrophils, epithelial cell, and bacterial cells are present; the arrow indicates bacterial cells, X 100.

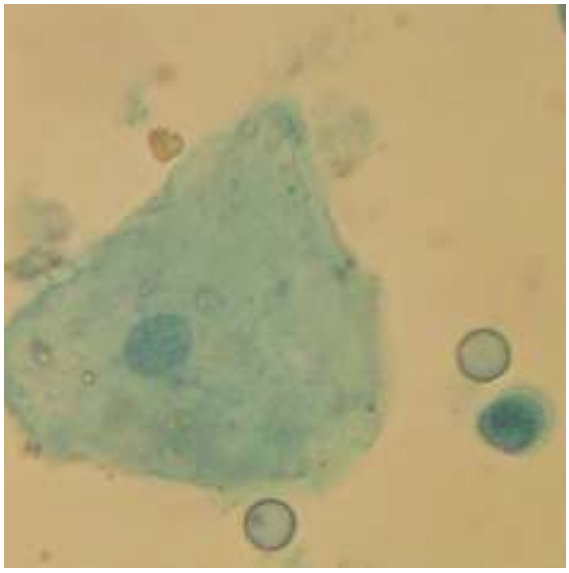

Figure (4)

Urine sediment smear stained with supravital Löeffler methylene blue stain. Neutrophil, erythrocytes, and epithelial cell are present; erythrocytes appeared with a homogenous disc surrounded with black line. X100.

Table – 1 –Color of the cells in both permanent smear which stained with (PSFSIP), and in wet mount smear which stained with löeffler methylene blue.

Cell / Stain	Neutrophil		Epithelial cell		Erythrocyte	Bacterial cell
	Cytoplasm	Nucleus	Cytoplasm	Nucleus		
PSFSIP				Dark bluish	Pink disc or crescent shape surrounded	Moderate

	Moderate blue.	Violet.	Pale blue.	violet.	by dark violet line.	to dark blue.
Löefflers methylene blue	Moderate greenish blue.	Dark greenish blue.	Pale greenish blue.	Moderate greenish blue.	Very pale blue surrounded by black line.	Dark greenish blue.

- One year follow up examination of 20 permanent stained smears of urine sediments, showed stability in shapes and colors of all the cells, for the whole period.

Discussion:

Microscopic analysis concentrates mainly on the diagnosis of cellular components of urine deposit, such as leukocytes, erythrocytes, epithelial cells, and casts, etc. These cells sometimes give a misleading

diagnostic feature when they are examined by direct examination within unstained smear[8]. In order to overcome this problem many techniques were devised. In 1956 esterase method was applied, this technique is used to detect leukocytes in urine by dipstick. The esterase released from the neutrophils reacts with ester on the reagent strip releasing 3-OH-5-phenyl pyrrole that reacts with diazonium salt to produce pink to purple color. The leukocyte esterase when compared with microscopy has sensitivity of 80% and specificity of 70% [1]. Staining technique, Permanent and supravital stains are also used as a prescreening tools for further, more extensive evaluation[8].

In this study, the PSFSIP stain was used as a permanent stain for fixed smears of urine sediments, the stained neutrophilic leukocytes in the smear of urine sediment, appeared with granulated moderate blue cytoplasm and violet segmented nucleus, while the epithelial cell appeared as a pale blue cytoplasm and round dark bluish violet nucleus. The contrast in color of cytoplasm and nucleus in each cell, made the shape and size of the nucleus appear very distinct. This characteristic feature is important in simplifying the process of differentiation between these two cells. Other investigators considered that the efficiency of the stain depends on its ability in reducing the confusion between neutrophilic leukocytes and epithelial cells [2, 11]. Erythrocytic cells appeared with pink color. This result was in agreement with other studies which reported that when red blood cells are stained may take up no stain or stained with light color [11]. In addition the normal and dysmorphic erythrocytes were clearly distinct in the stained smear of urine sediment. This characteristic is useful. Brich and Fairely [16] reported that microscopic examination for RBCs is used as a diagnostic test, distinguishing between hematuria of glomerular origin and hematuria of urinary tract . RBC's of glomerular origin were dysmorphic, in contrast to RBC's of non glomerular origin.

It is well known and as indicated by same studies that permanent staining technique provides a permanent record for pathological cases and also may be use for consultation with specialists when unusual morphologic characteristics shown in the smear[12, 13].

The permanent stain applied in this study showed good stability and efficiency in morphology and color intensity during the follow up examination for permanent smear along the entire period of one year, also this stain has a simple procedure, rapid, safe, and economic.

In conclusion, and according to the results of this study, it can be suggested that "Permanent stain for fixed smears of some intestinal parasites" is efficient in staining the cellular components of the urine sediment.

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