Quantitative Analysis of Urine Sediment Using Newly Designed Centrifuge Tubes

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Abstract. We quantified the formed elements of urine sediment using newly designed plastic centrifuge tubes with top and bottom openings and a 0.5 ml sized bottom ball (YZ tube). This design minimizes the adherence of formed elements that occurs on the glass surface of conventional tubes. The numbers of white blood cells (WBC) and red blood cells (RBC) using glass tubes did not differ from those observed using YZ tubes. However, the YZ tube method detected renal casts more frequently than the conventional glass tube method; the detection rate for renal casts in normal urine samples was 21.4% vs 2.9%, in samples from hospitalized patients it was 47.5% vs 10.2%, and from patients with kidney disease it was 88.9% vs. 44.4%. Especially, the YZ tube method detected more hyaline casts in all types of samples. The correlation between the glass tube and YZ tube methods was good for WBC (r=0.996), RBC (r=0.964), and epithelial cell count (r=0.939), but the correlation was weak for casts (r=0.511 for hyaline casts; r=0.359 for other casts). In conclusion, the YZ tube method of urine sediment analyses is an easy and accurate quantitative method; it is recommended as the method of choice for detecting and quantifying pathological casts in urine. (received 28 July 2001, accepted 28 September 2001)

Keywords: urine sediment, urinalysis, renal casts, YZ centrifuge tube

Introduction

Microscopic examination of urine sediment is a noninvasive, inexpensive, and often essential diagnostic test in patients with various diseases. In patients with hematuria, recognizing the site of bleeding is very important for both diagnosis and treatment [1-3]. Microscopic examination of urine sediments for pathological casts remains the most accurate diagnostic test to determine whether the source of hematuria is in the glomeruli or elsewhere in the genitourinary tract [3-5]. Even in large commercial laboratories, the detection rate of pathological casts is very low [6]. Such misleading reports may result in inaccurate diagnoses, improper management, and unnecessary investigations, such as intravenous pyelography, cystoscopy, and retrograde ureterography in acute and chronic glomerulonephritis [6].

Since there is no specific, standardized method for urine sediment microscopy, the normal reference values are variable. In many laboratories, the sediment in the bottom of glass centrifuge tubes is poured on slides in order to detect and quantify the formed elements of urine sediment. However, because the cells and casts in urine are not usually fixed, and since casts are formed of the protein matrix, they easily adhere to the glass surface of the tube. Additionally, mucus threads, which frequently occur in urine in large amounts, may trap the cells or casts [7]. Therefore, counting cells or casts in the poured urine samples can be falsely low.
In this study, we quantified the formed elements of urine samples using newly designed plastic centrifuge tubes with top and bottom openings and a 0.5 ml sized bottom ball. The sediments were taken directly through an opening on the bottom of the tubes.

**Methods and Materials**

**Specimens.** Random urine samples submitted to the urinalysis laboratory of St. Mary’s Hospital were used. The samples were from 253 patients (107 male, 146 female) with ages from 1 to 82 yr (median 46 yr). Nine of the patients had documented renal diseases. Seventy urine samples from normal adults (40 male, 30 female) were used as normal controls.

**Urinalysis Protocol.** Each urine sample was well-mixed and divided into two aliquots, 7 ml in a glass tube and 10 ml in a newly designed plastic tube with top and bottom openings and a 0.5 ml sized bottom ball (“YZ tube™”, Yzlab Inc., Korea) (Fig.1). To assess the precision obtained with the new tube, 80 urine samples were also divided into two YZ tubes. All tubes were centrifuged at 1500 rpm for 5 min. The urine in each glass tube was poured out and, with 0.3 to 0.4 ml of urine remaining, the sediment was suspended and poured onto a glass slide. The YZ tubes were mixed using a vortex mixer to suspend the sediment in the 0.5 ml of urine in the ball. (Because the ball on the bottom is covered with a cap, agitation with the analyst’s fingers was insufficient to suspend formed elements). The bottom cap was unscrewed and the first few drops of urine were dropped onto a glass slide.

Unstained sediments were examined by bright-field microscopy using 10x (low power field, lpf) and 40x (high power field, hpf) objectives. The same type of cover glasses (22x22 mm) was used in both methods. Both slides were examined by two experts blindly. The counts of WBCs, RBCs, and epithelial cells were reported as number of cells/hpf; the counts of casts were reported as number of casts/10 lpf. When needed, one drop of Sternheimer-Malbin stain was added (21 samples) or Papanicolaou stain was used (9 samples) to confirm the presence of epithelial cells or epithelial cell casts.

**Statistical analysis.** The blind results of urine sediment analysis and the results of dipstick chemical tests were tabulated, and statistical analyses were performed using the SPSS program. Correlation analysis of the conventional glass tube results and YZ tube results was performed using Pearson’s correlation test, and the differences were then analyzed using the paired-sample T test. The differences in the numbers of formed element and the results of chemical analyses were analyzed using the Mann Whitney U-test or the Chi-square test; p values <0.05 were considered significant.
Results

The number of WBC using the YZ tube method was 6.0±14.6 cells/hpf, and was not different from results by the conventional glass tube method (7.1±17.6). The number of RBC using the YZ tube method was 8.3±21.5 cells/hpf, and was also not different from results by the conventional glass tube method (8.8±19.6). All the control samples from normal adults revealed <5 WBC or RBC/hpf. However, the number of epithelial cells (3.8±5.6 cells/hpf) found using the YZ tube method was slightly higher than that using the conventional glass tube method (3.4±5.2, p=0.001) (Table 1).

Epithelial cell clumps (renal tubular cells or urothelial cells) were found in 12 of 253 samples using the conventional glass tube method and in 20 of 253 samples using the YZ tube method (Fig. 2). The number of hyaline casts (6.4±18.0 casts/lpf) and other casts (4.5±15.8 casts/lpf) using the YZ tubes was greater than found using conventional glass tubes (0.7±5.3 and 1.8±7.6, p <0.001). In some samples, numerous casts were mingled with mucus threads or among each other (Fig. 3). The numbers of WBC, RBC, epithelial cells, and casts in the urine were similar upon repeated examinations using YZ tubes (p >0.05).

Table 1. Comparison of the results of urine sediment analyses using the conventional glass tube method and the YZ tube method in all urine samples (n=323).

<table>
<thead>
<tr>
<th>Formed element</th>
<th>Glass tube method</th>
<th>YZ tube method</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC/hpf</td>
<td>7.1±17.6</td>
<td>6.0±14.6</td>
<td>ns</td>
</tr>
<tr>
<td>RBC/hpf</td>
<td>8.8±19.6</td>
<td>8.3±21.5</td>
<td>ns</td>
</tr>
<tr>
<td>Epithelial cells/hpf</td>
<td>3.4±5.2</td>
<td>3.8±5.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Hyaline casts /10 lpf</td>
<td>0.7±5.3</td>
<td>6.4±18.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other casts/10 lpf</td>
<td>1.8±7.6</td>
<td>4.5±15.8</td>
<td>0.004</td>
</tr>
</tbody>
</table>

hpf, high power field; lpf, high power field
The conventional glass tube method detected casts in 2 of 70 normal samples (2.9%), in 25 of 244 samples from hospitalized patients (10.2%), and in 4 of 9 samples from patients with kidney disease (44.4%). The YZ tube method detected casts in 15 of 70 normal samples (21.4%), in 116 of 244 samples from hospitalized patients (47.5%), and in 8 of 9 samples from patients with kidney disease (88.9%) (Table 2). The YZ tube method did not detect casts in 1 sample from a patient with kidney disease who suffered from acute glomerulonephritis and showed gross hematuria. Another sample with gross hematuria showed a RBC cast using the YZ tube method (Fig. 4). The YZ tube method detected more hyaline casts in all kinds of samples (Table 2).

The correlation between the conventional glass tube method and the YZ tube method is shown in Table 3. The WBC, RBC, and epithelial cell counts using both methods were well-correlated (r >0.9). The numbers of casts also correlated significantly with each other, but the correlation coefficients were only 0.51 for hyaline casts and 0.36 for other casts.

Discussion

The cells and pathological casts in urine are very important for diagnosing and monitoring patients with various diseases. However, the detection rate of pathological casts is very low in many laboratories [6]. Furthermore, the analysis of urine sediment is not standardized and quantitation of the formed elements in urine is troublesome. The YZ tubes are able to hold 10 ml of urine and are constructed with a 0.5-ml ball at the bottom and a downward opening. The urine is concentrated 20-fold by centrifuging and vortexing. The casts and mucus threads in the urine can easily exit the ball through the opening at the bottom.

Small numbers of RBC and WBC(<5/hpf) can be found in normal urine [7,8]. In this study, none of the normal control samples showed more than 5/hpf of WBCs or RBCs. The WBC and RBC counts assayed in all samples by the conventional glass tube method and the YZ tube method were not significantly different. Renal tubular epithelial cells are the most significant types of epithelial cells found in urine because the presence of an increased number of these cells in urine indicates tubular damage such as acute tubular necrosis and toxicity from certain drugs or heavy metals [8]. The presence in urine of

Table 2. The number of samples showing casts using the conventional glass tube method and the YZ tube method.

<table>
<thead>
<tr>
<th></th>
<th>Normal controls</th>
<th></th>
<th>Hospital patients</th>
<th></th>
<th>Kidney disease patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=70)</td>
<td>(n=244)</td>
<td>(N=9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass tube</td>
<td>YZ tube</td>
<td>Glass tube</td>
<td>YZ tube</td>
<td>Glass tube</td>
<td>YZ tube</td>
</tr>
<tr>
<td>Hyaline casts</td>
<td>2 (2.9%)</td>
<td>13 (18.6%)</td>
<td>5 (2.0%)</td>
<td>44 (18.0%)</td>
<td>1 (11.2%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Hyaline + other casts</td>
<td>0 (0.0%)</td>
<td>2 (2.9%)</td>
<td>2 (0.8%)</td>
<td>52 (21.3%)</td>
<td>0 (0.0%)</td>
<td>7 (77.8%)</td>
</tr>
<tr>
<td>Other casts</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>18 (7.4%)</td>
<td>20 (8.2%)</td>
<td>3 (33.3%)</td>
<td>1 (11.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (2.9%)</td>
<td>15 (21.4%)</td>
<td>25 (10.2%)</td>
<td>116 (47.5%)</td>
<td>4 (44.4%)</td>
<td>8 (88.9%)</td>
</tr>
</tbody>
</table>

Table 3. The correlation between the conventional glass tube (GT) method and the YZ tube (YZ) method for each formed element of urine.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT) WBC vs (YZ) WBC</td>
<td>0.996*</td>
</tr>
<tr>
<td>(GT) RBC vs (YZ) RBC</td>
<td>0.964*</td>
</tr>
<tr>
<td>(GT) epi cell vs (YZ) epi cell</td>
<td>0.939*</td>
</tr>
<tr>
<td>(GT) hyal cast vs (YZ) hyal cast</td>
<td>0.511*</td>
</tr>
<tr>
<td>(GT) other cast vs (YZ) other cast</td>
<td>0.359*</td>
</tr>
</tbody>
</table>

* p <0.001.
large clumps of transitional cells, in the absence of instrumentation, has clinical significance. The number of epithelial cells and epithelial cell clumps were found in more samples using the YZ tube method than using the conventional glass tube method. This may be explained by the fact that epithelial cells or clumps, which are large in size, tend to adhere to the surface of glass tubes.

Cellular and noncellular casts are formed in renal tubules and collecting ducts and are the only formed elements of urine that have the kidney as the sole site of origin. Casts vary in appearance, size, shape, and stainability. This variability may be one factor in the apparent low precision of cast identification in some laboratories [3,9]. In a normal person, a few hyaline casts can be seen in urinary sediment (0-2/lpf). Hyaline casts are the most frequently observed casts in normal persons, consisting almost entirely of Tamm-Horsfall protein [10]. The conventional glass tube method detected hyaline casts in only 2 of 70 normal samples (2.9%), while the YZ tube method detected hyaline casts much more frequently (15 of 70 normal samples, 21.4%). Two of these samples also revealed finely granular casts. Both coarsely granular and cellular casts are considered to be pathological, but finely granular casts can also be found in nonpathologic conditions [7,11-13].

The YZ tube method detected casts much more frequently in samples of normal controls, hospitalized patients, and patients with kidney disease than the conventional glass tube method. Since the YZ tube is made of plastic, and the formed elements are taken through the opening on the bottom, it is easy to get particularly large casts and cell clumps. In some samples, numerous casts were intermingled with mucus threads. In this situation, the YZ tube is also advantageous. Neither the conventional glass tube nor the YZ tube methods detected casts in one sample from a patient with kidney disease, who suffered from acute glomerulonephritis and showed gross hematuria. It is difficult to detect casts in specimens with gross hematuria. Another sample with gross hematuria showed RBC casts using the YZ tube method.

Increased numbers of casts usually indicate that renal disease is widespread and that many nephrons are involved [8]. Therefore, both the detection of pathological casts and the accurate quantitative report of their number are important. For quantitative analysis, cells and casts from undiluted, well-mixed or centrifuged urine sediments resuspended in a fixed volume of urine are counted and reported as the number of cells per microliter. This is a time-consuming process and the results are often variable [14]. Therefore, normal or reference values for formed elements vary from one laboratory to another. In this study, the YZ tube method showed good reproducibility. Using the YZ tube method, it is easier to quantify formed elements by centrifuging and vortexing, which may help to standardize the method of urine sediment analysis.

In conclusion, the YZ tube method of urine sediment analysis is an easy and accurate quantitative method; it is superior to the conventional glass tube method in the detection of casts and epithelial cell clumps. It is recommended as the method of choice in detecting and quantifying pathological casts in urine.

Acknowledgments

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References

5. Favaro S, Bonfante L, D’Angelo A, Giacomini A,


