Urinalysis and Body Fluids

Unit 3
Chemical Examination of Urine

Part 4, Urinary Proteins

Urine protein

- Small amounts of low-molecular weight protein are filtered at the glomerulus
  - Albumin has molecular weight @ 69,000 daltons
- Most of this protein is reabsorbed in the tubules
- Less than 150 mg/24 h (or 20 mg/dL) is excreted
- Proteinuria - abnormal / increased amounts of protein in the urine.
  - *Most important single indicator of renal disease - with good microscopic
- Mucoprotein Tamm-Horsfall is secreted by the renal tubules is also excreted
- Urine may also contain proteins from prostatic, seminal, and vaginal secretions.

Urine protein: significance

<table>
<thead>
<tr>
<th>Physiology causes (transient proteinuria)</th>
<th>Pathologic causes</th>
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</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>Glomerular nephritis</td>
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<tr>
<td>causes renal vasoconstriction</td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Emotional stress</td>
<td>Malignant hypertension</td>
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<tr>
<td>Exposure to heat or cold</td>
<td>↑ permeability of glomerulus</td>
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<tr>
<td>Fever</td>
<td>Tumors, SLE, infections, diabetes</td>
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<tr>
<td>Pregnancy</td>
<td>Disturbance of reabsorption</td>
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<tr>
<td>Orthostatic/postural</td>
<td>Others:</td>
</tr>
<tr>
<td>Common in teens when rapid growth is occurring</td>
<td>Systemic disorders, drugs, chemicals</td>
</tr>
</tbody>
</table>
Chemical Exam of Urine - protein

• Proteinuria in pregnancy
  • Pre-eclampsia and Eclampsia
    • Another very important reason for testing urine for protein is for early identification of pregnant women who are eclamptic.
    • worst form of “toxemia of pregnancy.”
    • Occurs in the latter stages of pregnancy in some women. 10% maternal mortality. 25% fetal mortality.
    • Complications include edema, hypertension, convulsions, coma, can lead to CVA, Pulmonary edema, renal failure, necrosis of the liver.
    • Early delivery of the fetus is indicated.
  • Pregnancy toxemia

Chemical Exam of Urine - protein

• Primary Renal Diseases
  • at one time called Bright’s disease after Richard Bright - doctor in 1827 used UA as part of office exam.
  • Kidney Diseases:
    • Specific objectives achieved: Four characteristics associated with Bright’s Disease
      • Nephritis - inflammation of the nephrons with hypertension, hematuria, increase in BUN, as well as proteinuria
      • Nephrotic syndrome - RBCs, cellular and granular casts and oval fat bodies
      • Loss of albumin from plasma, results in edema
      • Degree of protein loss has important prognostic meaning
      • Hypertension

Chemical Exam of Urine - protein

• Types of Protein
  • Serum proteins
    • Albumin
      • Easiest to slip through glomerulus
      • Most likely in kidney disease
      • Detected by dipstick
      • Precipitates in SSA
    • Tamm-Horsfall protein
      • Secreted by renal tubules
      • Not found anywhere else in the body.
      • Matrix material of casts
      • Not detected by the dipstick.
      • Precipitates in SSA
Chemical Exam of Urine - protein

- Types of Protein
  - Bence-Jones protein
    - From malignant clone of antibody producing cells
    - Low molecular weight (~44,000 daltons) passes easily
    - Made up of light chains (kappa or lambda)
    - Thermal sensitivity - when heated will coagulate at 40-60 degrees C (60) and re-dissolves at boiling (100 degrees C)
    - Found in 50-80% cases of multiple myeloma - strong assoc.
    - Bence-Jones protein does not read on dipstick (to any large degree) - dipstick specific for albumin
    - Testing for Bence-Jones proteinuria not part of routine UA but the protein may be detected in a back-up protein test.

Chemical Exam of Urine - protein

- Correlation of protein to microscopic
  - Casts
    - Tamm-Horsfall protein doesn't react on the dipstick,
    - However the same process that would cause increased cast production, usually results in proteinuria.
    - So if dipstick is positive for protein, look out for casts, and if you find numerous casts in a microscopic, may want to recheck dipstick result - a QC item.

Chemical Exam of Urine - protein

- Correlation of protein to microscopic
  - White cells and bacteria
    - Without protein usually indicates lower tract infection
    - With protein can indicate only kidney involvement or simultaneous upper (kidney) and lower tract infections
  - RBCs
    - Large amount will cause positive protein reading
Chemical Exam of Urine - protein

- **Methods of Testing**
  - Precipitation / coagulation tests
    - Use an acid to precipitate proteins (all types)
  - 3% sulfosalicylic acid
    - Most used
    - Detects any kind of protein
    - Added to the supernatant
    - Urine will turn cloudy if protein is present.

Chemical Exam of Urine

- **Principle:** "protein error of indicators"
- **Indicator** – tetrabromphenol blue
  - Most common
  - Can be hard to read at the trace end
- **Citrate Buffer** – maintains pH 3 - quite acid
  - Color ranges from yellow to blue
  - Detects primarily albumin
    - Not serum globulins, Tamm Horsfall or Bence Jones
  - Negative does not rule out other significant proteins

Urine Protein testing - Sources of Error

<table>
<thead>
<tr>
<th>False Positives</th>
<th>False Negatives</th>
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</thead>
<tbody>
<tr>
<td>Highly buffered / alkaline urine</td>
<td>Dilute urines</td>
</tr>
<tr>
<td>Prolonged exposure of dipstick in the urine</td>
<td>Elevated proteins other than albumin</td>
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<tr>
<td>Quaternary ammonium compounds&lt;br&gt;Cleaning agents alter pH</td>
<td></td>
</tr>
<tr>
<td>Phenazopyridine&lt;br&gt;Anesthetic medication&lt;br&gt;Used with antibiotics treat UTIs&lt;br&gt;Makes urine dark orange / red</td>
<td></td>
</tr>
<tr>
<td>Plasma expander polyvinylpyrrolidone</td>
<td></td>
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<tr>
<td>Chlorhexidine gluconate (skin cleansers)</td>
<td></td>
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<tr>
<td>Blood</td>
<td></td>
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<td>Anything that stains the dipstick</td>
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</table>
Chemical Exam of Urine

- Other means of measuring urine proteins
  - Urine protein electrophoresis
    - Concentrated specimen is placed onto a membrane and placed into an electrophoretic field
    - Different protein fractions migrate at different rates
    - Used to identify abnormal protein fractions, such as Bence Jones
  - Classical Bence Jones protein method
    - Filter urine while it is boiling hot
    - As it cools - watch for precipitation to form when temp @ 45-55 C
  - Quantitative Protein Tests (require 24 hour specimen)
    - Kjeldahl method - classical, reference method for measurement of protein
    - Kingsbury method - biuret reagent
      - An accurate measurement of urine protein output
      - Another alternative - urine protein to creatinine ratio

Reference Listing

- Please credit those whose work and pictures I have used throughout these presentations.
- Lillian Mundt & Kristy Shanahan, Greff's Textbook of Urinalysis and Body Fluids, 2nd Ed.
- Susan Strassinger & Marjorie Di Lorenzo, Urinalysis and Body Fluids, 5th Ed.
- Wikipedia, the free encyclopedia
  - www.wikipedia.org
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  - http://wwwmedicine.uiowa.edu/cm/cia/modules.asp?testID=19