Estimation of Serum Creatinine, Urine Creatinine, and Creatinine Clearance

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Lecture Overview

Estimation of Creatinine

Evaluation of Kidneys Function
- Creatinine production
- GFR
- Correlation between GFR, serum Cr. and Cr. Cl.

Experiments
- 1-Estimation of Serum Creatinine
- 2-Estimation of Urine Creatinine
- 3-Calculation of Creatinine Clearance
Evaluation of Kidneys Function

• Renal function tests are used to detect the presence of renal diseases and assess their progress. They are, however, of little use in determining the causes of renal disease.

• The most widely used test is to measure the glomerular filtration rate (GFR), that is, the rate of filtrate formation by the kidneys.
Creatinine production

- Creatinine is a substance that, in health, is easily excreted by the kidney. It is the byproduct of muscle energy metabolism and is produced at a constant rate according to the muscle mass of the individual.

- Endogenous creatinine production is constant as long as the muscle mass remains constant.5
What is the Glomerular filtration rate (GFR)?

- *The GFR is determined* by measuring the concentration of a substance in the urine and plasma that is known to be completely filtered from the plasma at the glomerulus.

- The GFR depend on the net [pressure](#) across the glomerular membrane, the [physical nature of the membrane](#) and it's [surface area](#) which in turn reflects the number of functional glomeruli.

- All three factors may be modified by disease, but in the absence of large changes in the filtration pressure or in the structure of the glomerular membrane, the GFR provides a useful index of the number of functional glomeruli. It gives an estimate of the degree of renal impairment by disease.
# Substances clearance used for Measuring GFR

<table>
<thead>
<tr>
<th>Source</th>
<th>Inulin Clearance</th>
<th>Creatinine Clearance</th>
<th>Urea Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td>(a polymer of fructose - plant carbohydrate)</td>
<td>End-product of skeletal muscle creatine metabolism</td>
<td>end product of protein metabolism</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Not reabsorbed or secreted</td>
<td>An endogenous product of muscle metabolism; near constant production</td>
<td>An endogenous product of protein</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Not made by body; must be injected</td>
<td>Small amount is secreted</td>
<td>• Partially reabsorbed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• synthesis varies with diet</td>
</tr>
</tbody>
</table>

- The Inulin is suitable for measuring GFR for the following reasons:
  - It is freely filterable by the glomeruli.
  - It is not reabsorbed or secreted by the kidney tubules.
  - It is not synthesized, destroyed, or stored in the kidneys
  - It is nontoxic
  - Its concentration in plasma and urine can be determined by simple analysis.
Creatinine production and protein intake must be assumed to be constant. Creatinine excretion is due not only to filtration (90%-95%) by the kidney but also to secretion (5%-10%) by the distal tubule.

FIGURE: Balance between muscle production and renal excretion of serum creatinine. As the glomerular filtration rate decreases, the percentage of creatinine excreted via secretion increases.
Correlation between serum creatinine and creatinine clearance

- The relationship between serum creatinine and creatinine clearance is logarithmic. Thus, initially, for small numeric changes in serum creatinine, there are significant numeric changes for creatinine clearance. In later stages of uremia, small numeric changes in the clearance are associated with significant changes in serum creatinine.
1-Estimation of Serum Creatinine

*High plasma creatinine*

Plasma creatinine tends to be higher in subjects with a large muscle mass. Other non-renal causes of increased plasma creatinine include the following:

- A high meat intake can cause a temporary increase.
- Transient, small increases may occur after vigorous exercise.
- Some drugs (e.g. salicylates) compete with creatinine for its tubular transport mechanism, thereby reducing tubular secretion of creatinine and elevating plasma creatinine.

*If non-renal cause can be excluded, an increased plasma creatinine indicates a fall in GFR, which can be due to pre-renal, renal or post-renal causes as follows*
2-Estimation of Urine Creatinine

**Decreased urine creatinine is found in:**

- a. Hyperthyroidism
- b. Anemia
- e. Inflammatory muscle disease
- f. Advanced renal disease, renal stenosis

**Increased urine creatinine is found in:**

- a. Diabetes mellitus
- b. Hypothyroidism
3- Estimation of Creatinine Clearance

The blood sample is sent to a lab. There, the creatinine level in the blood sample is tested. The lab specialist combines your creatinine level with several other factors to estimate your (GFR). Different formulas are used for adults and children. The formula includes some or all of the following: Age, Blood creatinine, Gender, Height and Weight

Interfering Factors

• 1. Exercise may increase creatinine clearance and urine creatinine.
• 2. Pregnancy substantially increases creatinine clearance.
• 3. A diet high in meat may elevate the urine creatinine concentration.
• 4. Proteinuria and advanced renal failure make creatinine clearance an unreliable method for determining GFR.
Clinical Implications

1. Decreased creatinine clearance is found in any condition that decreases renal blood flow:
   - a. Impaired kidney function
   - b. Shock, dehydration
   - c. Hemorrhage
   - d. Chronic obstructive lung disease
   - e. Congestive heart failure

2. Increased creatinine clearance is found in:
   - a. State of high cardiac output
   - b. Pregnancy
   - c. Burns
   - d. Carbon monoxide poisoning
Reference Values

Urine creatinine: 1-2 g/24h

(serum) creatinine: 0.6–1.2 mg/dL

*Normal creatinine clearance* = 100-130 ml/min/1.73m²
Objective

1- To estimate creatinine in serum and urine.
2- To calculate creatinine clearance value.

Principle:

(Jaffe’s method):
Colorimetric estimation of creatinine using the alkaline picrate method.

Creatinine + picric acid $\rightarrow$ Creatinine picrate (orange)
Absorbance at 520nm
Materials

A. Chemicals:
- Serum standard (3mg/dl)
- Urine standard (0.75mg/dl)
- Serum Sample
- Urine Sample
- Picric acid
- 2.5 M NaOH
- Dis.H2O

B. Instruments:
- Pipette with different volume capacity
- Cuvette
- Water bath
- Test tubes
- Alommunium foil
- Vortex
- S espectrophotometer
Method:
1- Set up a series of test tube as follows:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Standard (serum)</th>
<th>Test (serum)</th>
<th>Standard (Urine)</th>
<th>Test (urine)</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Standard (serum)</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum Sample</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Urine)</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urine Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Picric acid</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>-</td>
</tr>
</tbody>
</table>

2- Immerse the Tubes carefully in the boiling water bath for 40 seconds.
3- Set another 8 test tube labeled A- D twice and transfer 4 ml of each tube into the new set.
4- Pipette 0.2 ml of NaOH to all tube
5- Let the tubes stand for 20 min.
6- Read the absorbance at 520 nm.
<table>
<thead>
<tr>
<th>Tube</th>
<th>Standard(serum)</th>
<th>Test (serum)</th>
<th>Test (urine)</th>
<th>Standard(Urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(C )</td>
<td>(A)</td>
</tr>
<tr>
<td>Absorbance at 520 nm</td>
<td>(D)</td>
<td>(A)</td>
<td>(B)</td>
<td>(C )</td>
</tr>
<tr>
<td>Average(Mean of Absorbance)</td>
<td>(D)</td>
<td>(A)</td>
<td>(B)</td>
<td>(C )</td>
</tr>
</tbody>
</table>
Calculation:

1- Serum creatinine =
(Mean Absorbance of Urine ÷ Mean Absorbance of Standard) X concentration of Serum standard
= ........ mg / dl

2- Urine creatinine =
(Mean Absorbance of Urine ÷ Mean Absorbance of Standard) X concentration of Urine standard X DF
= ........ mg / dl

To compare with normal range, convert from mg/dl to g/24 h

3- Creatinine Clearance :=
\[ \frac{U \cdot V}{P} \]

U is Urine creatinine
V is Volume of urine in 24 h e.g 100 ml in 1440 min.
P is Serum creatinine
Creatinine Clearance

Find the Creatinine Clearance = if you know that the Urine creatinine $U = 488 \text{ mg/dl}$, Serum creatinine $P = 2.32 \text{ mg/dl}$, Volume of urine in 24 h $= 100 \text{ ml}$ and $A = 1.6 \text{ m}^2$

3- A- Creatinine Clearance: $= \frac{U \times V}{P}$

For example: $U = 100 \text{ ml/24 h}$

$\frac{(488 \text{ mg/dl} ÷ 2.32 \text{ mg/dl}) \times (100 ÷ 1440)}{14.6 \text{ ml/min}}$ = $14.6 \text{ ml/min}$

$14.6 \text{ ml/min}$ in $1.73 \text{ m}^2$ find Cr.Cl in this person who have a surface area $= 1.6$

$= \frac{(14.6 \times 1.6)}{1.73} = 13.5 / \text{min/1.73m}^2$

3- B- Creatinine Clearance: $= \frac{(U \times V \times 1.73)}{(P \times 1440 \times A)}$

$(488 \text{ mg/dl} \times 100 \times 1.73) / (2.32 \times 1440 \times 1.6)$

$= 15.7 \text{ ml/min /1.73m}^2$
Discussion

• Comment on the concentration of creatinine in serum.
• Comment on the concentration of creatinine in urine.
• Comment on the value of Creatinine Clearance.
Questions?

A man aged 35 years presenting loin pain has a serum creatinine of 150µmol/L. A 24 h urine of 2160 ml is collected and found to a creatinine concentration of 7500 µmol/L

Calculate the creatinine Clearance
References

- **Lecture Notes: Clinical Biochemistry** Geoffrey Beckett, Simon W. Walker, Peter Rae.
- **A Manual of Laboratory and Diagnostic Tests**, By Frances T Fischbach RN, BSN, MSN By Lippincott Williams & Wilkins Publishers.