Estimation of Serum Creatinine, Urine Creatinine, and Creatinine Clearance

BCH 472
Kidney functions:

- The kidneys serve three essential functions:
  1. They function as filters, removing metabolic products and toxins from the blood and excreting them through the urine.
  2. They regulate the body’s fluid status, electrolyte balance, and acid-base balance.
  3. The kidneys produce or activate hormones that are involved in erythrogenesis, Ca\(^{2+}\) metabolism, and the regulation of blood pressure and blood flow.
• **Renal function tests** are used to detect the presence of renal diseases and assess their progress.

• The most widely used test is to measure the **glomerular filtration rate** (GFR), that is, the rate of filtrate formation by the kidneys.
Glomerular Filtration Rate:

- Under **normal** conditions, approximately 625 mL of plasma flow through the kidneys each minute and the volume of plasma **filtered** is 125 mL/min which is called the glomerular filtration rate.

- **Glomerular filtration rate (GFR)**, is the **volume of plasma filtered by the kidneys in per unit of time**.

- **GFR** is an important and the best overall measurement in the evaluation of **kidney function**.
Measuring the GFR:

- Accurate measurement of the GFR by clearance tests requires determination of the concentration, in plasma and urine, of a substance is known to be completely filtered from the plasma at the glomerulus.

- This substance must not be reabsorbed nor secreted by renal tubules, broken down, or accumulated by the tubules and must remain at a constant concentration in the plasma throughout the period of urine collection.

- It's clearance is given by, $\text{clearance} = \frac{U \cdot V}{P}$
- (U= urine creatinine, p= plasma creatinine, v= volume of urine)
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>Non-toxic fructose polymer</td>
<td>Non-toxic fructose polymer</td>
<td>End-product of skeletal muscle creatine metabolism</td>
<td>endproduct of protein Metabolism</td>
</tr>
<tr>
<td>Advantages</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>Disadvantages</td>
<td>Not made by body; must be injected</td>
<td>Small amount is secreted</td>
<td>Partially reabsorbed synthesis varies with diet</td>
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</tbody>
</table>

* Creatinine clearance is preferred because it is a normal constituent of blood and **no infusion** is needed unlike inulin. Moreover it is **not reabsorbed** by the tubules as in the case of urea.
Creatinine:

- In the muscles “creatinine” is converted to creatine phosphate which becomes the source of a high energy phosphate bond for the immediate reformation of ATP.

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

- Endogenous creatinine production is constant as long as the muscle mass remains constant.
**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.

* If non-renal cause can be excluded, an **increased** plasma creatinine indicates a **fall in GFR**
Urine Creatinine

** Decreased urine creatinine is found in:**
- There are many cases leads to a decrease in urine creatinine eg. Advanced renal disease, renal stenosis.

** Increased urine creatinine is found in:**
There are many cases eg. Diabetes mellitus
Creatinine clearance:

A measure of the amount of creatinine eliminated from the blood by the kidneys.

- Creatinine is cleared from the body fluids almost entirely by glomerular filtration (small amount is secreted by kidney tubules).
- Therefore, the clearance of creatinine can be used to assess GFR.

- Because measurement of creatinine clearance does not require intravenous infusion into the patient, this method is much more widely used than inulin clearance for estimating GFR clinically.

- Tubules, to variable degree, secrete creatinine, which, by itself, would lead to an ~20% overestimate of GFR in humans.
Clinical Implications:

1. **Decreased creatinine clearance** is found in any condition that decreases renal blood flow:
   - Impaired kidney function.
   - Shock, dehydration.
   - Hemorrhage.

2. **Increased creatinine clearance** is found in:
   - Pregnancy
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

Note: What 1.73 m² means?
Kidney function is proportional to kidney size, which is proportional to body surface area. A body surface area of 1.73 m² is the normal mean value for young adults.

Adjustment for body surface area is necessary when comparing a patient’s estimated GFR to normal values or to the levels defining the stages of CKD.
Practical Part

Experiments

1- Estimation of Serum Creatinine

2- Estimation of Urine Creatinine

3- Calculation of Creatinine Clearance
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 → Creatinine picrate (orange)
Absorbance at 520nm
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>
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</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
<td>(D)</td>
<td>(E)</td>
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<td>Water</td>
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<td>2 ml</td>
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<td>Standard (serum)</td>
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<td>Serum Sample</td>
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<td>0.5 ml</td>
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<tr>
<td>Standard (Urine)</td>
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<td>-</td>
<td>-</td>
<td>0.5 ml</td>
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<tr>
<td>Urine Sample</td>
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<td>0.5 ml</td>
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<td>0.5 ml</td>
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<tr>
<td>Picric acid</td>
<td>6 ml</td>
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</table>
2- Immerse the Tubes carefully in the boiling water bath for 40 seconds.
4- Pipette 0.6 ml of NaOH to all tube
5- Let the tubes stand for 20 min.
6- Read the absorbance at **520 nm**.

### Results:

<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>(D)</td>
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<tr>
<td><strong>Absorbance at 520 nm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Average (Mean of Absorbance)</strong></td>
<td></td>
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</tbody>
</table>
Calculation:

1- Serum creatinine =
(Mean Absorbance of sample serum ÷ Mean Absorbance of Standard serum) X concentration of standard serum (3 mg/dl) = ............ mg / dl

2- Urine creatinine =
(Mean Absorbance of sample urine ÷ Mean Absorbance of Standard) X concentration of standard urine (0.75 mg/dl) X DF (100) = ........... mg / dl

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

3- Creatinine Clearance = \( \frac{U.V}{P} \)
= \( \frac{\text{Urinary creatinine (mg/dl)}}{\text{plasmac reatinine (mg/dl)}} \) x Urine volume (ml/min) = A

Note: 24h urine volume = 100ml, body surface = 1.6 m²

- A------------------------→ 1.6 m²
- ?------------------------→ 1.73 m²
- Corrected for surface area = ml/min/1.73 m²
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 $V = 100 \text{ ml}$ and $A$ (surface area) = $1.6 \text{ m}^2$

**A- Creatinine Clearance: $= \frac{U \cdot V}{P}$**

For example: $V = 100 \text{ ml/24 h}$ >> to convert hours to minutes $\frac{1}{1440}$ “24x60”

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

14.6 ml/ min in **1.6 m²**, find Creatinine clearance in **1.73m²**:

$=(14.6 \times 1.73) \div 1.6 = 15.8 \text{ ml/min}/1.73\text{m}^2$

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

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

$= 15.8 \text{ ml/min}/1.73\text{m}^2$
Discussion:

- Comment on the concentration of creatinine in serum.
- Comment on the concentration of creatinine in urine.
- Comment on the value of Creatinine Clearance.
Question-Home work:
A man aged 35 years has a serum creatinine of 3 mg/dl. A 24 h urine of 2160 ml is collected and found to a creatinine concentration of 400 mg/dl
Calculate the Creatinine Clearance