ESTIMATION OF SERUM CREATININE, URINE CREATININE AND CREATININE CLEARANCE

BCH 472 [Practical]

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 (RFT)

- 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.

3

Glomerular Filtration Rate (GFR)

- 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 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.

- **Clearance is given by:**

Clearance (ml/min) = U.V/ P

➔Where:

U= concentration of any substance in urine.

P= concentration of the same substance in plasma.

V= volume of urine (ml/min).

5

Substances used for Measuring GFR (clearance test)

	Inulin Clearance	Creatinine Clearance	Urea Clearance
Source	Non-toxic fructose polymer.	End-product of skeletal muscle creatine metabolism.	End-product of protein metabolism.
Advantages	Not reabsorbed or secreted.	An endogenous product of muscle metabolism; near constant production.	An endogenous product of protein.
Disadvantages	Not made by body; must be injected (exogenous).	Small amount is secreted.	-Partially reabsorbed. -Synthesis varies with diet.

Creatinine

- **Creatinine** is derived from "creatine" which is synthesized in the liver, kidney and pancreas it moves through the circulation and is taken up entirely by muscles.
- Creatinine is a substance that, in health, is easily excreted by the kidney.
- It is the by-product 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.

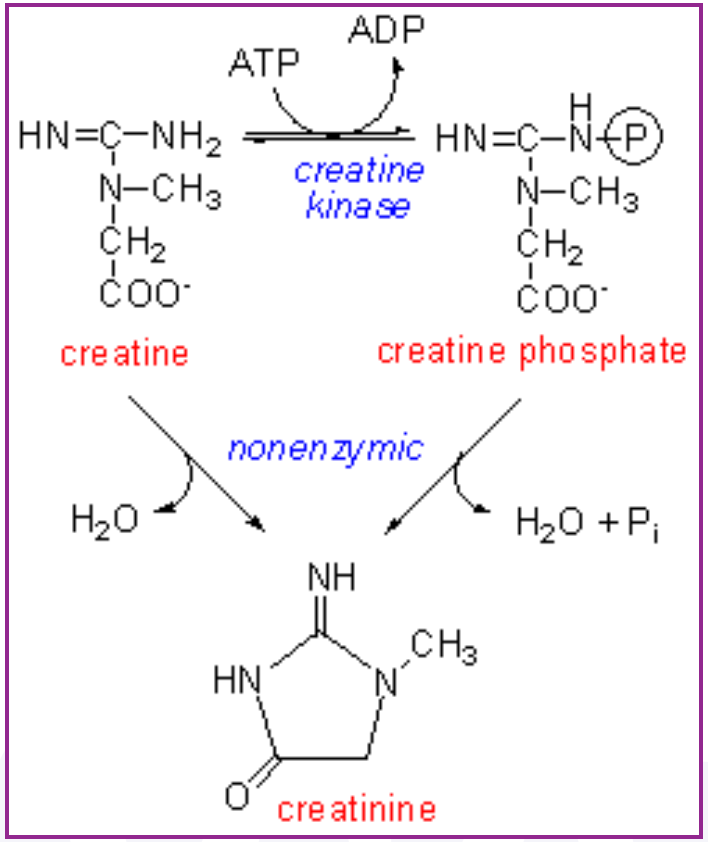

Figure 1. Creatinine reaction

Creatinine clearance

- A measure of the amount of creatinine eliminated (filtered) 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.

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 (renal disease).

Urine Creatinine

Decreased urine creatinine is found in:

- Advanced renal disease.
- Renal stenosis.

Increased urine creatinine is found in:

- Diabetes mellitus.

Clinical Implications of creatinine clearance

Decreased creatinine clearance is found in any condition that decreases renal blood flow:

- Impaired kidney function.
- Shock, dehydration.
- Haemorrhage.
- Hypothyroidism

Increased creatinine clearance is found in:

- Pregnancy.
- Hyperthyroidism

Reference Values

- Urine creatinine :1- 2 g/24h
- Serum creatinine: 0.6–1.2 mg/dL
- Creatinine clearance: 100-130 ml/min/1.73m^2

Note:

What 1.73 m^2 means?

- Kidney function is proportional to kidney size, which is proportional to body surface area. A of 1.73 m^2 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 GFR criterion for the diagnosis of CKD, and to levels defining the stages of CKD.

Chart 1. Chronic kidney disease staging

Stage	Description	GF (ml/ min/1.73m²)
I	Kidney lesion with normal or increased GF	≥ 90
II	Kidney lesion with mild GF decrease	60-89
III	Kidney lesion with moderate GF decrease	30-59
IV	Kidney lesion with marked GF decrease	15-29
V	Functional kidney failure or undergoing SRT	< 15

SRT- substitutive renal therapy. Source: National Kidney Foundation, 2002.

Practical Part

Objectives:

- To estimate creatinine in serum and urine.
- 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 8 test tube as follows:

Chemical	Standard (3mg/dl)		Test (serum)		Test (urine)		Blank
	(A)	(B)	(C)	(D)	(E)	(F)	
Water	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	2 ml
Standard	0.5 ml	0.5 ml	-	-	-	-	-
Serum Sample	-	-	0.5 ml	0.5 ml	-	-	-
Urine Sample	-	-	-	-	0.5 ml	0.5 ml	-
Picric acid	6 ml	6 ml	6 ml	6 ml	6 ml	6 ml	6 ml

2- Immerse the Tubes carefully in the boiling water bath for 40 seconds.

3- Pipette 0.6 ml of NaOH to all tube.

4- Let the tubes stand for 20 min.

Results:

Tube	Standard		Test (Serum)		Test (Urine)	
	(A)	(B)	(C)	(D)	(E)	(F)
Absorbance at 520 nm						
Average (Mean of Absorbance)						

Calculation

Patient information: 24h urine volume = 100ml, gender: women, body surface: 1.6m^2, DF=100.

1- Serum creatinine=

(Mean Absorbance of serum test ÷ Mean Absorbance of Standard) X concentration of standard = …… mg / dl

2- Urine creatinine=

(Mean Absorbance of Urine test ÷ Mean Absorbance of Standard) X concentration of standard X **DF**= …… mg / dl

(To compare with normal range, convert from mg/dl to g/24 h) ***HOW?***

3- Creatinine Clearance:

=U.V/ P

= [($\boldsymbol{Urinary\ creatinine\ (mg/dl)}$) / ($\boldsymbol{plasmac\ creatinine\ (mg/dl)}$)] $\boldsymbol{x\ Urine\ volume(ml/min)}$ = B

B ----------> 1.6 m^2 (person surface area)

? ----------> 1.73 m^2

Corrected for surface area= **…….. ml/min/1.73 m^2**

Example:

Find the Creatinine Clearance if you know that the Urine creatinine **U = 488 mg/dl** , Serum creatinine **P= 2.32** mg/dl, Volume of urine in **24 h =100 ml** and A "surface area"=1.6 m^2 ?

➔ Creatinine Clearance: = U.V/ P

= (488 mg/dl ÷2.32 mg/dl) × (100÷1440*)= 14.6 ml/min

14.6 ml/ min in 1.6 m2, find the creatinine clearance for 1,73 m2 surface area :

=(14.6 ×1.73)÷1.6= **15.8 ml /min/1.73m2**

--------OR-------

➔ Creatinine Clearance: = (U XV X1.73)/ (P X 1440 X A)

= (488 mg/dl× 100× 1.73)/ (2.32× 1440× 1.6)

= **15.8 ml/min /1.73m^2**

* To convert 24 hour to min (24x60 = 1440)

Discussion

- Comment on the concentration of creatinine in serum and urine.
- Comment on the value of Creatinine Clearance.

Homework

A man aged 40 years with surface area of 1.8m^2 has a serum creatinine of 6 mg/dl. A 24 h urine of 2200 ml is collected and the urine creatinine concentration found to be 450 mg/dl.

Calculate the Creatinine Clearance.

References

- Kidney function tests: MedlinePlus Medical Encyclopedia. (n.d.). Retrieved from https://medlineplus.gov/ency/article/003435.htm
- (N.d.). Retrieved from https://www.mayoclinicproceedings.org/article/S0025-6196(11)60609-5/fulltext
- 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.