LAB : 8

(RENAL FUNCTION TESTS)

Renal function, in nephrology, is an indication of the state of the kidney and its role in renal physiology. Glomerular filtration rate (GFR) describes the flow rate of filtered fluid through the kidney. Creatinine clearance rate (C_{Cr} or CrCl) is the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the GFR. Both GFR and C_{Cr} may be accurately calculated by comparative measurements of substances in the blood and urine.

Kidney function tests is a number of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys can help determine the cause and extent of kidney dysfunction. These tests are done on urine samples, as well as on blood samples.

Physiology:

The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain between (15-40) mg/dl.
The most common cause of an elevated B.Urea(azotemia) is poor kidney function, although a serum creatinine level is a somewhat more specific measure of renal function .A greatly elevated BUN (>60 mg/dL) generally indicates a moderate-to-severe degree of renal failure. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves.

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a substance secreted by the liver, and removed from the blood by the kidneys

A high BUN value can mean:

1-kidney disease

2-Blockage of the urinary tract (by a kidney stone or tumor)

3- Low blood flow to the kidneys caused by dehydration or heart failure.

4-Many medicines may cause a high BUN.

5-A high BUN value may be caused by a high-protein diet, tissue damage (such as from severe burns), or from bleeding in the gastrointestinal tract.

A low BUN usually has little significance:

1) Liver problems.
2) Malnutrition (insufficient dietary protein).
3) Excessive alcohol consumption.
4) Over hydration .
5) During pregnancy.

Kidney functions :

-Regulation of water and electrolyte balance.

-Regulation of acid base balance.

-Regulation of arterial blood pressure.

- Excretion of metabolic waste products and foreign chemicals.

-Metabolic Function : site for gluconeogenesis.

Renal diseases :

- Many diseases affect renal function.
- In some, several functions are affected.
- In others, there is selective impairment of glomerular function or one or more tubular function .

Signs and Symptoms of Renal Failure

- Symptoms of Uraemia (nausea, vomiting, lethargy)
- Disorders of Micturition (frequency, nocturia, dysuria)
- Disorders of Urine volume (polyuria, oliguria, anuria)
- Alterations in urine composition (haematuria, proteinuria, bacteriuria, leukocytouria, calculi)
- Pain
- Oedema (hypoalbuminaemia, salt and water retention)

Why Tests of Renal Function are Important?

- To identify renal dysfunction.
- To diagnose renal disease.
- To monitor disease progress.
- To monitor response to treatment.
- To assess changes in function that may impact on therapy (e.g. Digoxin, chemotherapy).

Blood tests:

There are several blood tests that can aid in evaluating kidney function. These include:

1-Blood urea nitrogen test (BUN).

2-Creatinine test.

3-Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

1- Blood Urea Nitrogen (BUN)

Urea is the characteristic and most abundant nitrogenous end product of protein catabolism in mammals. It is generated by the liver and excreted by the kidney. Urea filters easily through the glomerulus into the ultra-filtrate. It will diffuse passively into the blood as it passes down the renal tubules. Under conditions of normal flow

and normal renal function, about 40% of the filtered urea is reabsorbed; when the flow rate is decreased, the amount passively reabsorbed increases. As with creatinine, the serum concentration of urea nitrogen rises with impaired renal function.

The serum concentration of urea nitrogen is influenced by factors not connected with renal function or urine excretion as it is affected strongly by the degree of protein catabolism. A marked change in dietary protein consumption will be reflected in BUN values. The injection or ingestion of steroids produces a rise in BUN as do stressful situations that cause the adrenal gland to secrete additional cortisol. For these reasons, the measurement of serum creatinine is a better indicator of kidney status than is that of BUN although in many cases, they go up and down simultaneously. The various **prerenal**, **renal**, and **postrenal** factors that affect creatinine also influence the BUN.

Clinical Significance

The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with a blood creatinine, a more specific indicator of kidney function.

Expected Values

Normal range in serum or plasma of BUN (20 – 45 mg/dL)

BUN / Creatinine Ratio

Normal BUN / Creatinine ratio is 12 – 20 to 1

2-Serum Creatinine

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Chemically, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys. 98% of the body creatine

is present in the muscles where it functions as store of high energy in the form of creatine phosphate.

About 1-2 % of total muscle creatine or creatine phosphate pool is converted daily to creatinine through the spontaneous, non enzymatic loss of water or phosphate.

Serum creatinine is a better indicator of renal function than either that of BUN or uric acid because is not reabsorbed by the renal tubules , creatinine is an endogenous substance not affected by diet and Plasma creatinine remains fairly constant throughout adult life.

Expected Results

Males = 0.6 – 1.4 mg /dl
Females = 0.5 – 1.2 mg /dl

- **Clearance** : Volume of plasma from which a measured amount of substance can be completely eliminated into urine per unit of time expressed in milliliters per minute.
- Function: Estimate the rate of glomerular filtration

Creatinine Clearance:

Used to **estimate** GFR (glomerular filtration rate) .Most sensitive measure of kidney function .Mathematical derivation taking into effect the serum creatinine concentration to the urine creatinine concentration over a 24- hour period.

- **Reference range**:
- **Male 97 - 137 mL/min**
  Female 8 -128 ml/min

What causes kidney failure?

Kidney failure can occur from an acute situation or from chronic problems.

In acute renal failure, kidney function is lost rapidly and can occur from a variety of insults to the body. The list of causes is often categorized based on where the injury has occurred.

Prerenal causes are due to decreased blood supply to the kidney. Examples of prerenal causes are:

- Hypovolemia (low blood volume) due to blood loss
- Dehydration from loss of body fluid (vomiting, diarrhea, sweating, fever
- Poor intake of fluids
- Medication, for example, diuretics ("water pills") may cause excessive water loss.
  - Increased Ratio- BUN is high/ creatinine is normal

Renal causes (damage directly to the kidney itself) include:

- Multiple Myeloma
- Acute glomerulonephritis or inflammation of the glomeruli, the filtering system of the kidneys. Many diseases can cause this inflammation.
- Systemic diseases (SLE-rheum. Arthritis)
  - Normal Ratio- both BUN and creatinine are **proportionally** elevated

Post renal causes are due to factors that affect outflow of the urine:

- Obstruction of the bladder or the ureters can cause back pressure when there is no place for the urine to go as the kidneys continue to work. When the pressure increases enough, the kidneys shut down.
- Prostatic hypertrophy or prostate cancer may block the urethra and prevents the bladder from emptying.
- Tumors in the abdomen that surround and obstruct the ureters.
- Kidney stones

  -Increased Ratio- BUN is high and creatinine also elevated.

Chronic renal failure develops over months and years. The most common causes of chronic renal failure are related to:

- Poorly controlled diabetes
- Poorly controlled high blood pressure
- Chronic glomerulonephritis

BUN: Methodology

- -**Kjeldahl** – a classical method for determining urea concentration by measuring the amount of nitrogen present
- -**Berthelot reaction** - Good manual method - that measures ammonia uses an enzyme (urease) to split off the ammonia
- -**Diacetyl monoxide** (or monoxime)

  Popular method but not well suited for manual methods. **Because** uses strong acids and oxidizing chemicals

Determination of BUN

Principle

Determination of urea is by the indirect method using the urease-modified Berthelot reaction. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide as in the following equation:

$$\textbf{Urea} + H_2O \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored dye the absorbance of which is proportional to the urea concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with 24 volume of R2. The working reagent is stable for 30 days at 2-8 °C

Working reagent	Sample	standard
1 ml	**10 µl**	**-----**
1 ml	**-----**	**10 µl**

Mix and incubate at 37 °C for 5 minutes. Then add

R3	**1ml**

Mix and incubate at 37 °C for 5 minutes. Measure the absorbance For the sample and standard at 600 nm.

Calculation:

$$\text{Concentration of BUN} = \frac{\text{Absorption of sample}}{\text{Absorption Standard}} \times \text{Conc of Stan}$$

Concentration of Standard is 50 mg/dl

Determination of Creatinine

Kinetic method (Modified Jaffe reaction):

Principle: Creatinine in protein-free filtrate of serum or diluted urine in alkaline solution reacts with picric acid to form a red-orange chromogen the absorbance of which is proportional to the creatinine concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with1 volume of R2. The working reagent is stable for 30 days at 2-8 °C.

Working reagent	Sample	standard
1 ml	**100 µl**	**-----**
1 ml	**-----**	**100 µl**

Mix gently. Then insert the test tube in to the instrument and start stop watch. Read the absorbance after 30 sec (A1) and after 90 sec (A2). Read at 510 nm.

Calculation

$$(A2 - A1)\ \text{sample} / (A2 - A1)\ \text{standard} \times \text{Conc of Stan}$$

Concentration of Standard is 2 mg/dl