SCHOOL OF MEDICNE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR

REVIEW RENAL FUNCTION

What are the Regulatory Functions of kidneys?

- **Regulation of Water / Fluid Balance**:
  - Arginine Vasopressin (AVP) {Anti-Diuretic Hormone} produced by Posterior Pituitary acts on Kidney Tubules causing Reabsorption of Water from Glomerular filtrate.
  - AVP stimulates incorporation of Aqua-porins into cell membranes in the collection duct where reabsorption of water occurs.

- **Regulation of Electrolyte Balance**:
  - Aldosterone acts on Kidney Tubules causing Reabsorption of Na^+ ions in exchange for secretion of K^+ ions and H^+ ions

- **Regulation of Acid-base balance**:
  - Kidneys involve in maintaining pH (acidity/alkalinity) in blood and other body fluids

- **Parathyroid Hormone** (**PTH**):
  - Promotes Tubular Re-absorption of Calcium;
  - Promotes Phosphate Excretion
  - Promotes Synthesis of 1, 25-Dihydroxy-Cholecalciferol, which regulates Calcium Absorption in Gastrointestinal Tract (GIT)

- **Renin**: An enzyme produced by Juxtaglomerular cells in kidneys
  - Renin catalyzes conversion of Angiotensinogen to Angiotensin-1
    - Angiotensin Converting Enzyme (**ACE**) then converts Angiotensin-1 to Angiotensin II
    - Angiotensin II stimulates biosynthesis of Aldosterone in Adrenal Cortex

- **Erythropoietin**:
  - Peptide hormone that promotes biosynthesis of Hemoglobin
  - Production of Erythropoietin is partly regulated by Kidneys

- **Endocrine effects of the kidneys remain intact until End Stage Renal Failure**

What are the Renal Function Tests

- Collective term for a variety of individual tests and procedures used to evaluate the Functional State of the kidney:
  - **Tests for Glomerular Functions**
  - **Tests for Tubular Functions**

- Renal Function Tests Include the following:
  - **Urinalysis**: First line test for Renal Function
  - **Creatinine Clearance (CC)**: used to measure Glomerular Filtration Rate (**GFR**)
  - **Inulin Clearance**: used to measure GFR
  - **Para-Amino-Hippuric Acid (PAH)**: used to measure Renal Plasma Flow (**RPF**)
  - Urine Osmolality, Plasma Creatinine, Plasma Urea, Plasma Electrolyte

What tests are involved in Urinalysis?

- Randomly collected Urine sample is examined:
  - **Physically** for: Color, Odor, Appearance, and Concentration (Specific Gravity) or Osmolarity
  - **Chemically** for: Protein, Glucose, and Urine pH (acidity/ alkalinity);
  - **Microscopically** for the presence of:
    - Cellular elements (RBC, WBC, Epithelial cells),
    - Bacteria, Crystals, Casts (structures formed by deposit of protein, cells, and other substances in the kidneys' tubules)

What is Glomerular Filtration Rate (GFR)?

- GFR is a useful index of the numbers of functioning Glomeruli
- GFR in the number of milliliters of filtrate made by the kidneys per minute
- GFR measures the rate at which the kidneys can clear a compound from the blood
- Normal GFR depends on normal Renal Blood Flow and Pressure
- GFR is directly related to body size, thus it is higher in men than in women
- GFR is also affected by age, with a reduced rate in elderly
- Reduction in GFR can be caused by:
  - Restriction of Renal blood supply,
  - Low Cardiac Output,
  - Destruction of Nephrons by Renal Diseases, etc
- Reduction in GFR results in Retention of Waste Products of Metabolism in blood

How can GFR be determined / calculated?

- GFR is directly related to Clearance,
- GFR can be calculated from the Clearance of some Plasma Constituent, which is freely Filtered at the Glomerulus, and is neither Reabsorbed nor Secreted by Kidney Tubules
- For Clinical Practice Creatinine, present in blood as a normal product of muscle metabolism, comes close to fulfilling the above requirements

- GFR can be calculated from the Creatinine content of a 24-hour urine collection, and the Plasma concentration of Creatinine within this period
- **Inulin** can be used to measure GFR because it is filtered but not re-absorbed or secreted by Renal Tubules

GFR is Calculated as follows: (Creatinine Clearance or Inulin Clearance)

- **GFR = CC = (U x V)/P**

  - ***Where U = Urine concentration of Creatinine (mmol/L)***
  - ***P = Plasma or Serum concentration of Creatinine (mmol/L or as μmol/L)***
  - ***V = Urine Flow Rate (ml/minute)***

- **{V** is Urine Flow Rate = Total Vol of Urine collected in 24-hours divided by **24 x 60**}

- GFR value must be corrected for body surface area of patients, this is calculated from the Age and Height of the patient in relation to the "Standard" Average Body Surface Area of normal individual **"Standard" average body surface area = 1.73m^2**

Using the Cockcroft and Gault formula to calculate Creatinine Clearance

- Cockcroft and Gault formula can be used to estimate the Creatinine Clearance of patients with the following exceptions:

  - Patients should not be severely malnourished
  - Patients should not be very obese
  - Renal Function should not be severely impaired (GFR < 20 ml/min)

$$\textbf{CC (ml/min)} \quad \frac{\textbf{(140 – Age in yrs) x Wt in Kg x (0.85 for a female)}}{\textbf{0.814 x Serum Creatinine (micromoles / L)}}$$

NB: To correct for muscle mass in **Male patients**: **Multiple results by 1.22**

What substance is used to measure Renal Plasma Flow (RPF)?

- Para-Amino-Hippuric Acid (PAH) is used to measure RPF because it is filtered and secreted by Renal Tubules

How is RPF calculated?

- RPF is calculated by the Clearance Equation

$$\mathbf{RPF = C_{PAH} = \frac{[U]_{PAH} \times V}{[P]_{PAH}}}$$

Where

C_{PAH} = Clearance of PAH;
$[U]_{PAH}$ = Urine concentration of PAH,
V = Urine Flow Rate,
$[P]_{PAH}$ = Plasma concentration of PAH

What is Proteinuria?

- Glomerular basement membrane does not usually allow passage of albumin and large molecular weight proteins
- Small amount of protein, usually less than 25mg/24h is found in urine
- Positive screening test for protein (included in a routine urinalysis) on a random urine sample is usually followed-up with a test on a 24-hour urine sample that more precisely measures the quantity of protein
- Protein, in excess of 250mg/24h urine sample indicates significant damage to Glomerular membrane
- Persistent presence of significant amounts of protein in the urine, is an indicator of kidney disease

What are the different types and causes of Proteinuria?

Glomerular Proteinuria:

- Abnormal leaking of large and small molecular weight proteins into Glomerular filtrate resulting from damaged of Glomerular membrane

- May be due to Exercise, Fever (Febrile Proteinuria), Congestive Cardiac Failure, Glomerulonephritis, Renal Stenosis

- **Glomerulonephritis:**
  - **Common cause of persistent Proteinuria**
  - Amount of protein in urine depends on:
    - Extent of Glomerular damage,
    - Molecular mass of protein,
    - Capacity of Tubule to reabsorb or metabolize the proteins

  - May be mild, moderate or Severe Proteinuria
  - Severe Proteinuria: Protein loss in urine exceeds synthetic capacity of body to replace protein, thus resulting in HypoProteinemia (low protein in blood)

- Severe persistent Proteinuria is one of the feature of Nephrotic Syndrome

- **Nephrotic Syndrome:**
  - Large amount of protein loss in urine
    - Leads to Hypo-Proteinemia and Edema
      - Edema may be caused by low albumin and secondary Hyper-Aldosteronism

    - Patients may also develop Hyper-Lipidemia

  - Causes of Nephrotic Syndrome: Glomerulonephritis, Systemic Lupus Erythematosus, Diabetes Nephropathy

Tubular Proteinuria:

- Failure of Tubules to reabsorb filtered plasma proteins
- Abnormal secretion of protein into urinary tract
  - May be due to Tubular or Interstitial damage

- Low with low molecular mass are excreted by Tubules
- Loss of protein usually mild about 2.0g/24hours
- Sensitive test for assessment of Renal Tubular damage:
- Measure Urinary β_2-Microglobulin (> 0.4mg/24hours indicates damage)

- **Overflow Proteinuria:**
  - Large amount of low molecular weight proteins accumulate the urine.
  - Proteins are filtered at the Glomerulus, but are not reabsorbed or catabolised completely by the Tubules

  - **Causes of Overflow Proteinuria**: Acute Pancreatitis, Multiple Myeloma, Intravascular Hemolysis, Myelomonocytic Leukemia, Crush Injuries

- **Orthostatic (Postural) Proteinuria:**
  - Proteinuria occurs after standing for a long time
  - Protein absent in early morning urine samples

Plasma Creatinine:

- Creatinine is a by-product of muscle energy metabolism
- Creatinine is cleared from the blood by the kidneys and excreted in urine
- Production of Creatinine depends on muscle mass, which fluctuates very little
- Normally Creatinine in blood remains relatively constant
- Plasma Creatinine concentration is inversely proportional to Creatinine Clearance
- Plasma Creatinine concentration is affected very little by Liver function,
- Elevated Plasma Creatinine is a sensitive indication of impaired Renal function
- Normal Plasma Creatinine concentration of a patient does not always indicate normal Renal function

- Progressive rise in serial Plasma Creatinine concentration indicates impaired Renal function

Blood Urea Nitrogen (BUN)

- Urea is a by-product of protein metabolism
- Urea is formed in the Liver, released in the blood then filtered by the Glomerulus and excreted in the urine
- BUN test measures the amount of Nitrogen contained in Urea
- High BUN levels can indicate kidney dysfunction, but because BUN is also affected by protein intake and liver function, the test is usually done in conjunction with blood creatinine, a more specific indicator of kidney function
  - Urea can be affected by other factors, thus elevated BUN, by itself, is suggestive, but not diagnostic, of kidney dysfunction

What other parameters in blood that can be measured:

- Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function.
- These include:
  - Electrolytes (Sodium, Potassium, Chloride, Bicarbonate),
  - Calcium, Magnesium, Phosphorus, Protein, Uric Acid, and Glucose

- **Urine Osmolality**:

  - Osmolality is the measurement of Urine concentration that depends on the number of particles dissolved in the urine
    - Osmolality is expressed as mOsm/kg (milli-Osmols per kilogram) of water
  - For "apparently" healthy person the **Urine** is usually more concentrated than **Plasma**

    - **Urine Osmolality / Plasma Osmolality > 1**

  - Measurement of Osmolality can be carried out on early morning urine samples, on multiple timed urine samples, or on a cumulative sample collected over a 24-hour period
  - Inability of the kidney to concentrate the urine in response to restricted fluid intake, or to dilute the urine in response to increased fluid intake may indicate decreased Renal Function

Renal Tubular Function:

- Tubular reabsorption must be efficient to ensure effective reabsorption of: Water, Sodium, Glucose, Bicarbonate, etc are not lost from the body
- About 180 litres of fluid pass into the Glomerular filtrate each day, and about 99% is reabsorbed via the Tubules

- Tubular function can be assess by comparing Osmolality of Urine and Osmolality of Plasma
- Urine / Plasma Osmolality Ratio is usually between 1.0 and 3.0, because Urine is normally more concentrated than Plasma

    - Urine / Plasma ratio < 1.0, indicates that Renal Tubules are not reabsorbing water

- Some disorders of Tubular function are inherited:
    - Example, Some patients are unable to reduce their urine pH below 6.5, because of a specific failure of Hydrogen ion secretion

How does the kidney regulate Acid-Base balance?

- Kidney regulates Acid-Base Balance by controlling:
    - Re-absorption of Bicarbonate ions (HCO_3^-)
    - Secretion of Hydrogen ions (H^+)

- Both processes depend on formation of HCO_3^- ions H^+ ions from CO_2 and H_2O within Renal Tubular cells:

$$\mathbf{CO_2 + H_2O \xleftrightarrow{\text{Carbonic Anhydrase}} H_2CO_3 \longleftrightarrow H^+ + HCO_3^-}$$

- H^+ ions formed are actively secreted into Tubule fluid in exchange for Na^+

What mechanisms are used in the kidney for elimination of Acids?

- Mechanisms used for elimination of Acids include:
    - **Re-absorption** of Sodium Bicarbonate ($NaHCO_3$) by Proximal Renal Tubules, **(Fig. 1)**
    - **Regeneration** of HCO^-_3 by Distal Renal Tubules (Fig. 2)
    - Production of Ammonia (NH_3) by Distal Renal Tubules, which secrete H^+ ions and maintain a gradient of H^+ ions between cell and lumen

    (Figs. 3 & 4)

- Secretion of H^+ ions by the Tubular cells serves initially to reabsorb HCO_3^- ions from the Glomerular filtrate so that they are not lost from the body
- When all the HCO_3^- ions have been recovered (reabsorbed), any deficit due to the buffering process is regenerated

How are the HCO_3^- ions in the Glomerular Filtrate Reabsorbed (Reclaimed or Recovered)? (Interpretation of Fig. 1)

- **HCO_3^- ions** are freely filtered by the Glomerulus
    - Amount of **HCO_3^-** ions in filtrate is equivalent to that in Plasma
- If **HCO_3^- ions** were not reabsorbed in Renal Tubules the Buffering Capacity of Blood Plasma would be depleted rapidly
- Reabsorption of **HCO_3^- ions** occur mainly in **Proximal Tubule**

- HCO_3^- ions filtered through the Glomerulus combine with H^+ ions secreted from the Tubular cell forming Carbonic Acid (H_2CO_3)
- H_2CO_3 is then converted to CO_2 and H_2O by Carbonic Anhydrase II, which is present in the brush border of Renal tubular cells

    - CO_2 produced readily crosses into Tubular cell

- Inside Tubular cell the CO_2 interacts with H_2O again, to form H_2CO_3 catalyzed by Carbonic Anhydrase II
- H_2CO_3 then dissociates to form HCO_3^- ions and H^+ ions

- HCO_3^- ions formed diffuse into the blood stream whilst the H^+ ions are transported into the Tubular Lumen in exchange for Na^+ ions
- About 80 – 90% of HCO_3^- ions in the Glomerular Filtrate are Reabsorbed or Reclaimed in Proximal Tubule

How are the HCO_3^- ions Regenerated?

- After reabsorption of HCO_3^- ions is completed, the process of regeneration compensates for any deficit in the amount of HCO_3^- reabsorbed
- Mechanisms for reabsorption of HCO_3^- ions and for the regeneration of HCO_3^- ions are completely different (**Compare Fig. 1 and Fig. 2**)

How are Hydrogen ions (H^+) excreted by the Renal Tubules?

- H^+ ions are secreted in exchange for Na^+ ions
- Energy for this exchange comes from Na^+ - K^+ -ATPase (Sodium-Potassium Pump) that maintains the concentration gradient for Na^+ ions
- H^+ ions are secreted by two major buffers:

    - Phosphate buffer ($H_2PO_4^-$ / HPO_4^{2-})

    - Ammonium buffer (NH_4^+ / NH_3)

- Phosphate (HPO_4^{2-}) is freely filtered by Glomerulus and passes down the Tubule where it combines with H^+ ions to form $H_2PO_4^-$ (**Fig. 3**)

- Ammonia (NH_3) is produced in Renal Tubular cells by the action of the enzyme **Glutaminase** on the Amino acid Glutamine

$$\text{Glutamine} + H_2O \text{ =======}\rightarrow \text{Glutamate} + NH_3$$

- Glutaminase functions optimally at a lower (more acidic) than normal pH
- More Ammonia is produced during Acidosis thus improving the buffering capacity of the Urine
- Ammonia is un-ionised and so rapidly crosses into the Renal Tubular Lumen down its concentration gradient
    - NH_3 combines with H^+ ions to form NH_4^+ ions (Ammonium ions), which being ionised does not pass back into the tubular cell

- NH_4^+ ions are lost in urine, along with H^+ ions (**Fig. 4**)