Urine and its Importance
(Urine analysis)

Javad Zavar Reza
Ph.D in Clinical Biochemistry
Department of Biochemistry
jzavar@ssu.ac.ir
Glomerular Filtration
Water, salts, nutrient molecules, and waste molecules move from the glomerulus to the inside of the glomerular capsule. These small molecules are called the glomerular filtrate.

Proximal convoluted tubule (PCT)
Glomerular capsule
Efferent arteriole
Urea
H₂O
Glucose
Amino acids
Salts
Gluconeogenesis

Tubular Reabsorption
Nutrient and salt molecules are actively reabsorbed from the proximal convoluted tubule into the peritubular capillary network, and water flows passively.

Distal convoluted tubule (DCT)
Drugs
H⁺

Tubular Secretion
Certain molecules are actively secreted from the peritubular capillary network into the distal convoluted tubule.

Reabsorption of sodium from the DCT and water from the collecting duct are under hormonal control.

Renal artery
Renal vein
Venules
Arterioles
Loop of the nephron
Peritubular capillary network
Collecting duct
H₂O
Salts
Urea
Uric acid
NH₄⁺
Creatinine
Collection of urine

- **Early morning sample- qualitative**
  - concentrated urine
  - chemical constituents
  - casts and crystals

- **Random sample- routine**
  - chemical screening
  - microscopic examinations

- **24 hour urine sample**
  - For qua sugars, electrolytes, and hormones
  - quantitative estimation of proteins
Collection of urine (Midstream urine specimen)

- Urinary Tract Infection (UTI)
- First 10 – 25 ml of urine is discarded
- It contains urethral and prostatic secretions which may be required if investigating urethra and prostate.
- Post prandial sample-D.M
Urine examination

- Macroscopic examination
- Chemical examination
- Microscopic
Macroscopic examination

- Volume: Normal = 600-1550ml
- Color
- Odour
- Reaction or urinary pH
- Specific gravity
Causes of polyuria

- Diabetes mellitus
- Diabetes insipidus
- Polycystic kidney
- Chronic renal failure
- Diuretics
- Intravenous saline/glucose

- Dehydration-
vomiting, diarrhoea,
sweating
- Renal ischemia
- Acute tubular necrosis
- Obstruction to the urinary tract
- Acute renal failure
Urinary pH/ reaction

-Reflects ability of kidney to maintain normal hydrogen ion concentration in plasma & ECF
  - Normal = 4.6-8

- Ketosis-diabetes, starvation, fever
- Systemic acidosis
- UTI- E.coli
- Acidification therapy
- Strict vegetarian
- Systemic alkalosis
- Urinary Tract Infection(UTI)
- Alkalization therapy
Biochemical examination
Tests for proteins

- The glumeruli act as an ultrafilter
- The degree filtration depend on MW, ionic charge and plasma concentration

- Normal  = 150 mg/day
- Greater : Proteinuria
Tests for proteins
(Colorimetric reagent strip test)

- **Trace**: 5 to 20 mg/dl.
- **Alkaline urine** and contamination with ammonium compounds or skin cleansers containing chlorohexidine may produce false results.
Microalbuminuria

- Alb is small and globular, so large amounts filtered into the glomerular urine, increased if acidic groups are blocked.

- **Mildly increased excretion** (20-300 mg/day) called microalbuminuria
Microalbuminuria

- The level of Alb produced by microalbuminuria cannot be detected by urine dipstick methods.
Significance of microalbuminuria

- An indicator of subclinical CVD
- An important prognostic marker for kidney disease
  - in diabetes mellitus
  - in hypertension
- increasing microalbuminuria during the first 48 hours after admission to an ICU predicts elevated risk for acute respiratory failure, multiple organ failure, and overall mortality
Causes of glycosuria

- **Glycosuria with hyperglycaemia** - diabetes, acromegaly, cushing’s disease, hyperthyroidism, drugs like corticosteroids.

- **Glycosuria without hyperglycaemia** - renal tubular dysfunction
# Ketone Body Accumulation in Diabetic Ketosis

<table>
<thead>
<tr>
<th></th>
<th>Urinary Excretion (mg/24h)</th>
<th>Blood Con. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>125 ≤</td>
<td>3</td>
</tr>
<tr>
<td>ketosis</td>
<td>5000</td>
<td>90</td>
</tr>
</tbody>
</table>
Ketones in urine (ketonuria)

- Diabetes mellitus,
- **Other Causes of Ketonuria:**
  - Fever
  - Anorexia
  - Gastrointestinal disturbances
  - Fasting
  - Starvation
  - Starvation
  - Sever
Bile in the Urine

- The constituents
  - bilirubin (bile pigments)
  - bile salts
  - urobilin and urobilinogen

- Liver diseases - injury, hepatitis

- Obstruction to biliary tract
Carbohydrate & Clinical Correlations
Diabetes Mellitus

Diagnosis

- Fasting Blood Sugar (FBS)
- Random Test
- 2 hour Postprandial glucose test
- Glucose Challenge Test (GCT)
- Glucose Tolerance Testing

Follow up

- HbA1c
Fasting Blood Sugar (FBS)

1. <100 mg/dL = Normal
2. 100-125 mg/dL = Impaired Fasting Glucose (IFG)
3. >126 mg/dL on two separate tests = Diabetes
Diabetes Mellitus (Diagnosis)
Random Test

- Casual is defined as any time of day without regard to time since last meal
- ≥ 200 mg/dl (11.1 mM) plus Symptoms of diabetes
  - Polyuria, polydipsia, unexplained weight loss
- Confirm on subsequent visit with fasting blood glucose or oral glucose tolerance test.
Diabetes Mellitus (Diagnosis)  
Glucose Challenge Test (GCT)

Sample drawn 1 hour after a 50-gram glucose drink. 1-hour glucose challenge

<table>
<thead>
<tr>
<th>Glucose Level</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 140* mg/dL (7.8 mmol/L)</td>
<td>Normal screen</td>
</tr>
<tr>
<td>140* mg/dL (7.8 mmol/L) and over</td>
<td>Abnormal,</td>
</tr>
</tbody>
</table>

* Some use a cutoff of 130 mg/dL (7.2 mmol/L) because that identifies 90% of women with gestational diabetes, compared to 80% identified using the threshold of 140 mg/dL (7.8 mmol/L).
Glucose Tolerance Testing

- For adults with impaired FBS during pregnancy if at risk

Procedure

- Following 8 hour fast
- Glucose dose = 1.75g/kg BW
  - Maximum 75 g dose (BW<43kg, 94lbs)
  - 25 g/dl glucose test solution
  - Test at 2 hours
Sample drawn after 100-gram glucose drink (glucose load).

<table>
<thead>
<tr>
<th>Time of Sample Collection</th>
<th>Target Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting* (prior to glucose load)</td>
<td>95 mg/dL (5.3 mmol/L)</td>
</tr>
<tr>
<td>1 hour after glucose load</td>
<td>180 mg/dL (10.0 mmol/L)</td>
</tr>
<tr>
<td>2 hours after glucose load</td>
<td>155 mg/dL (7.8 mmol/L)</td>
</tr>
<tr>
<td>3 hours after glucose load*</td>
<td>140 mg/dL (7.8 mmol/L)</td>
</tr>
</tbody>
</table>

**INDICATION:** If two or more values meet or exceed the target level, gestational diabetes is diagnosed. IGT if [glu] > 140 - 199 at 2 hours

*A 75-gram glucose load may be used, although this method is not as well validated as the 100-gram OGTT; the 3-hour sample is not drawn if 75 grams is used.*
Diagnostic Criteria

- DM if $[\text{glu}] \geq 200 \text{ mg/dl}$ at 2 hours
- IGT if $[\text{glu}] > 140 - 199 \text{ at 2 hours}$
- Random Test: Normal $< 140 \text{ mg/dl}$
- IGT and IFT recently termed pre diabetic

*IGT: Impaired glucose tolerance
Diabetes mellitus

- FBS at or above 126 mg/dL (7.0 mmol/L),

- Two-hour value in an GTT (2-h PG) at or above 200 mg/dL (11.1 mmol/L)

- Random plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L) in the presence of symptoms.

- The diagnosis of diabetes must be confirmed on a subsequent day by measuring FBS, or 2-h PG, or random plasma glucose (if symptoms are present).
Follow up of Diabetes mellitus

HbA1c: the blood test with a memory
## Correlation Between Hemoglobin A1c and Mean Plasma Glucose Levels

<table>
<thead>
<tr>
<th>Hemoglobin A1c (%)</th>
<th>Approximate mean plasma glucose (mg/dL)</th>
<th>Approximate mean plasma glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>65</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>5.5</td>
</tr>
<tr>
<td>6</td>
<td>135</td>
<td>7.5</td>
</tr>
<tr>
<td>7</td>
<td>170</td>
<td>9.5</td>
</tr>
<tr>
<td>8</td>
<td>205</td>
<td>11.5</td>
</tr>
<tr>
<td>9</td>
<td>240</td>
<td>13.5</td>
</tr>
<tr>
<td>10</td>
<td>275</td>
<td>15.5</td>
</tr>
<tr>
<td>11</td>
<td>310</td>
<td>17.5</td>
</tr>
<tr>
<td>12</td>
<td>345</td>
<td>19.5</td>
</tr>
</tbody>
</table>
Serum Protein & Clinical Correlations
BLOOD

Plasma = Less Dense

Platelets / WBC’s
Hematocrit
“Packed Cells”
More Dense
Serum protein electrophoresis

- Albumin
- $\alpha_1$
- $\alpha_2$
- $\beta$

- Concentration 65–83 g/L
- Simple or conjugated (glycoproteins, lipoproteins)
# Elfo fractions of plasma proteins

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Rel. amount (%)</th>
<th>C (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Albumins</strong>: albumin</td>
<td>52 – 58</td>
<td>34 – 50</td>
</tr>
<tr>
<td>pre-albumin (transthyretin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>α&lt;sub&gt;1&lt;/sub&gt;-globulins</strong>: thyroxin-binding globulin, transcortin, α&lt;sub&gt;1&lt;/sub&gt;-acid glycoprotein, α&lt;sub&gt;1&lt;/sub&gt;-antitrypsin, α&lt;sub&gt;1&lt;/sub&gt;-lipoprotein (HDL), α&lt;sub&gt;1&lt;/sub&gt;-fetoprotein</td>
<td>2,4 – 4,4</td>
<td>2-4</td>
</tr>
<tr>
<td><strong>α&lt;sub&gt;2&lt;/sub&gt;-globulins</strong>: haptoglobin, macroglobulin, ceruloplasmin</td>
<td>6,1 – 10,1</td>
<td>5 – 9</td>
</tr>
<tr>
<td><strong>β-globulins</strong>: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system</td>
<td>8,5 – 14,5</td>
<td>6 – 11</td>
</tr>
<tr>
<td><strong>γ-globulins</strong>: IgG, IgM, IgA, IgD, IgE</td>
<td>10 – 21</td>
<td>8 – 15</td>
</tr>
</tbody>
</table>
ALBUMIN

- Concentration in plasma: 45 g/L
- ~ 60% of the total plasma protein

Functions:
- maintenance of the osmotic pressure of plasma
- transport of:
  - steroid hormones
  - free fatty acids
  - bilirubin
  - drugs (sulfonamides, aspirin)
  - Ca^{2+}, Cu^{2+}
Albumin Deficiency

- Liver diseases (cirrhosis) – decrease in the ratio of albumin to globulins
- Protein malnutrition
- Excessive excretion by kidneys (renal disease)
- Mutation causing analbuminemia (affects splicing)
Hypoproteinemia

- Causes: malnutrition, nephrotic syndrome, Severe starvation, severe chronic disease, Protein-losing enteropathy.
- Decreases in all fractions (most Alb. 20 g/L).
- The very large molecules are retain.
Hypoproteinemia
Protein-losing enteropathy

- Decreased albumin
- Decreased gamma globulins
- Increased $\alpha_2$-macroglobulin
Specific loss of proteins into the urine

- occurs on a molecular weight basis.
- Nephrotic syndrome
- Glomerular proteinuria
- AMG, HDL
- Tubular proteinuria(2)
Nephrotic Syndrome

- Decreased albumin
- Increased $\alpha_2$-macroglobulin
- Decreased gamma globulins

[Diagram showing protein peaks labeled as Albumin, $\alpha_1$, $\alpha_2$, $\beta$, $\gamma$.]
Decreased albumin (synthesis)
Increased gamma globulins (polyclonal gammopathy)

- The loss of Alb is balanced by marked polyclonal increase in Igs (IgA) with a γ–fraction
- Igs contribute significantly to oncotic pressures.
Tests for Evaluation of Liver Function
Liver Function tests

- May measure synthetic function
- May measure excretory function
- May indicate damage to cells: determination
- Of specific enzymes may be used to show the
- Location of liver damage
Liver Function tests

Classified in 3 groups

- Synthetic function: albumin, PT
- Hepatocyte injury: AST, ALT
- Cholestasis: bilirubin, ALP, GGT

- PT, albumin, bilirubin-most common tests used as prognostic factors
### Liver Function Test

<table>
<thead>
<tr>
<th>Liver chemistry test</th>
<th>Clinical implication of abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>Hepatocellular damage</td>
</tr>
<tr>
<td>AST</td>
<td>Hepatocellular damage</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Cholestasis, impair conjugation, or biliary obstruction</td>
</tr>
<tr>
<td>ALP</td>
<td>Cholestasis, biliary obstruction</td>
</tr>
<tr>
<td>PT</td>
<td>Synthetic function</td>
</tr>
<tr>
<td>Albumin</td>
<td>Synthetic function</td>
</tr>
<tr>
<td>GGT</td>
<td>Cholestasis or biliary obstruction</td>
</tr>
<tr>
<td>Bile acids</td>
<td>Cholestasis or biliary obstruction</td>
</tr>
<tr>
<td>5'-nucleotidase</td>
<td>Cholestasis or biliary obstruction</td>
</tr>
<tr>
<td>LDH</td>
<td>Hepatocellular damage, not specific</td>
</tr>
</tbody>
</table>
Hepatic Neoplasm Markers

- **AFP:** < 25 µg/L, > 500 µg/L: primary hepatocellular carcinoma
- **CEA:** 0-5 µg/L, increased CEA: liver metastatic carcinoma or other carcinomas of the gastrointestinal system
- **Abnormal prothrombin Time (APT):** increased APT: primary hepatocellular carcinoma
Clinical utility of Urea & Creatinine
BUN

- BUN: Blood Urea Nitrogen
  
  • BUN is an old term, but still in common use
  • Specimen: Plasma or serum

  • convert urea to BUN in mg/dL by using following formula: 
    \[ \text{BUN} = \frac{\text{Urea}}{2.14} \]
  • MW of urea = 60 urea nitrogen
    So 2.14 = 60/28
Synthesed in the kidneys and liver
Transported to muscle and brain
1 to 2% of muscle creatine spontaneously converts to creatinine daily and released into body fluids at a constant rate.

Endogenous creatinine produced is proportional to muscle mass, it is a function of total muscle mass the production varies with age and sex.
Clinical utility of Creatinine

Released at a constant rate ➔ its clearance is indicator of GFR.

Small quantity is reabsorbed by the tubules and other quantities are actively secreted by the renal tubules ➔ So its clearance is approximately 7% greater than inulin clearance.

The difference is not significant when GFR is normal but when the GFR is low (less 10 ml/min), tubular secretion makes the major contribution to Cra.

So excretion the Cra. clearance significantly overestimates the GFR.
Plasma creatinine

- Plasma Cra. is inversely related to the GFR.
- But GFR can decrease by 50% before plasma Cra. rises beyond the normal range. **Normal plasma Cra. does not necessarily imply normal renal function.**
- Raised Cra. usually indicates impaired renal function.
- Changes in plasma Cra. can occur, independently of renal function, due to changes in muscle mass.
- Cra is partially secreted by the proximal tubules via the organic cation pathway, and is blocked by various drugs including cimetidine, trimethoprim and salicylate.
Azotemia = Elevated plasma BUN

- The key point: ↑ plasma urea without ↑ plasma creatinine.

Prerenal ↑ BUN

- Low Blood Pressure (CHF, Shock, hemorrhage, dehydration)
- Decreased blood flow to kidney = No filtration
- Increased dietary protein or protein catabolism

Prerenal ↓ BUN

- Decreased dietary protein
- Increased protein synthesis (Pregnant women, children)
BUN disease correlations

- **Renal** causes of ↑ BUN
  - Renal disease with decreased glomerular filtration
    - Glomerular nephritis
    - Renal failure form Diabetes Mellitus
  - Post renal causes of ↑ BUN
    - Obstruction of urine flow
      - Kidney stones
      - Bladder or prostate tumors
      - UTIs
BUN / Cra Ratio

- **Normal**: BUN mg:Cr mg = 12-20:1
- **Lower ratio**
  - Low protein intake, starvation, liver disease
- **Higher ratio with normal Cra.**
  - Tissue breakdown, pre renal azotemia, high protein intake, GI hemorrhage
- **Higher ratio with elevated Cra.**
  - Post renal obstruction or prerenal azotemia superimposed on renal disease.
Blood Tests to Detect Inflammation
**Erythrocyte sedimentation rate**

- The ESR measures the rate at which the RBC separate from the plasma and fall to the bottom of a test tube. The rate is measured in (mm/hr).

  If certain proteins cover red cells, these will stick to each other and cause the red cells to fall more quickly.

**Men under 50 years old:** < 15 mm/hr  
**Men over 50 years old:** < 20 mm/hr  
**Women under 50 years old:** < 20 mm/hr  
**Women over 50 years old:** < 30 mm/hr
Acute Phase Reactants

Other ACPs include $\alpha_1$-acid glycoprotein, haptoglobin, and ceruloplasmin
C-reactive protein

- The reference range for C-reactive protein is as follows:
  - CRP: 0-10mg/L
  - High-sensitivity CRP (hs-CRP): < 1 mg/L
Thyroid hormone
Anterior pituitary hormones

- Hypothalamic hormones: PRH, TRH, CRH, GHIH, GHRH
- Anterior pituitary hormones: Prolactin, TSH, ACTH, GH, FSH, LH
- Endocrine targets: Thyroid gland, Adrenal cortex, Liver, Many tissues
- Nonendocrine targets: Breast, Germ cells of the gonads
- Neurons secreting trophic hormones

- Portal system: Hypothalmus, Anterior pituitary, Endocrine cells
- To target tissues: Thyroid hormones, Cortisol, IGFs, Androgens, Estrogens, progesterone
## TABLE 24.11
Characterization of Thyroid Disorders According to Results of Thyroid Function Tests

| Disorder                        | TSH | T<sub>4</sub> | T<sub>3</sub> | FT<sub>4</sub> | Tg | TBG | rT<sub>3</sub> | aTPO | ATG | TBI | TSI | TBA |
|---------------------------------|-----|--------------|--------------|--------------|----|-----|-----------|------|-----|-----|-----|-----|-----|
| Primary hypothyroidism          | ↑   | ↓            | N or ↓       | N or ↓       | N  | ↓   | N or ↑    | N or ↑| N or ↑|     |     |     |
| Transient neonatal hypothyroidism | ↑   | ↓            | ↓            | ↓            | N  | ↓   | N         | N    | ↑    |     |     |     |
| Hashimoto’s thyroiditis/hypothyroidism | ↑   | N or ↓      | N or ↓       | N or ↓       | N  | ↓   | ↑         | ↑    | n or ↑|     |     |     |
| Graves’ disease                 | ↓   | ↑            | ↑            | ↑            | ↑  | ↑   | ↑         | ↑    | ↑    |     |     |     |
| Neonatal Graves’ disease        | ↓   | ↑            | ↑            | ↑            | ↑  | ↑   | N         | n or ↑| n or ↑|     |     |     |
| TSH deficiency                  | N  | N or ↓      | ↓            | ↓            | ↓  | ↓   | N         | n    | n    |     |     |     |
| Thyroid dyshormonogenesis        | ↑   | ↓            | ↓            | ↓            | N  | ↓   | N         | n    | n    |     |     |     |
| Thyroid hormone resistance      | N  | ↑            | ↑            | ↑            | ↑  | ↑   | N         | n    | n    |     |     |     |
| TSH-dependent hyperthyroidism    | ↑   | ↑            | ↑            | ↑            | ↑  | ↑   | N         | n    | n    |     |     |     |
| T<sub>4</sub> protein-binding abnormalities* | N | V             | V             | N             | N  | V+  | V         | n    | n    |     |     |     |
| Nonthyroidal illness             | V  | N or ↓      | ↓            | V            | N  | N   | N or ↑    | n    | n    |     |     |     |
| Subacute thyroiditis†            | ↓ or ↑ | ↑ or ↓ | ↑ or ↓ | ↑ or ↓ | ↑ or ↓ | ↑ or ↓ | N | ↑ or ↓ | n |     |     |     |
Depiction of the evolution of primary hypothyroidism of autoimmune origin