Clinical biochemistry

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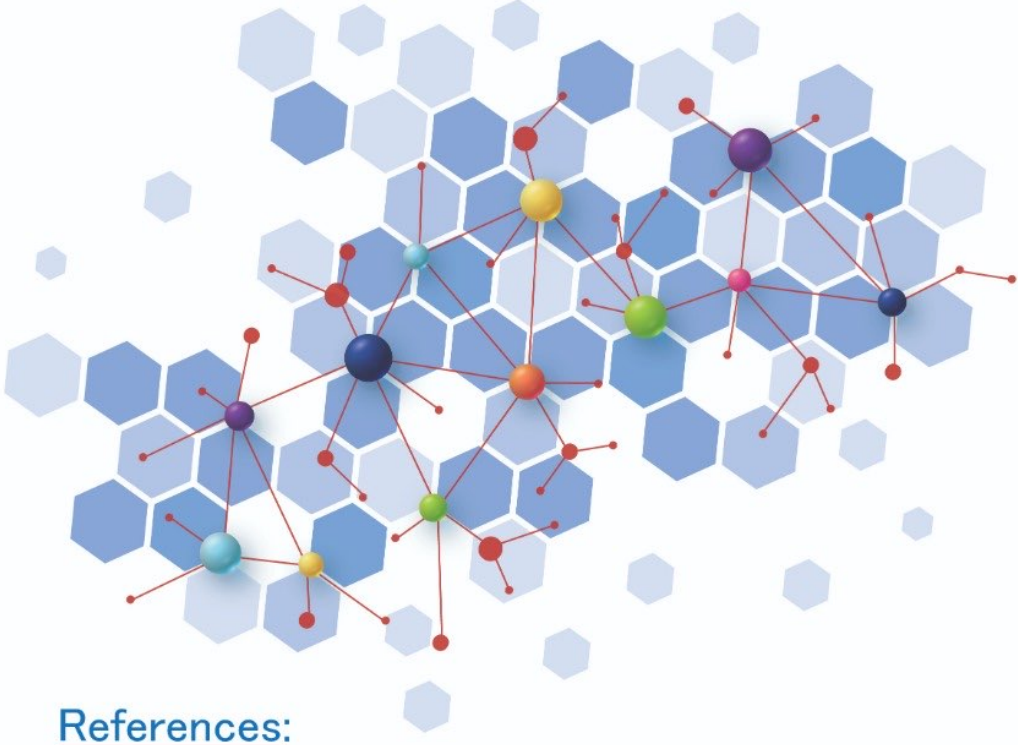


References:

- Clinical biochemistry book 13th edition.
- Doctor's slides and records.
- Past years questions.

Version: 1

Lecture #: #1: Biochemical Investigations

Biochemical investigation in clinical medicine

Test	Result	Reference
CBC		
RBC (× 10^6/µl)	6.15	5.0–11.20
Haemoglobin (g/dl)	10.0	10.6–15.6
Haematocrit (%)	34.4	31.7–48.0
MCV (fl)	55.9	36.7–55.0
MCH (pg)	16.3	12.3–17.3
MCHC (g/dl)	29.1	30.1–35.6
RDW (%)	18.3	16.7–22.9
Platelets (× 10^3/µl)	323	175–500
WBC (× 10^3/µl)	4.13	4.04–18.70
Chemistry – Canine/feline standard panel		
Glucose (mg/dl)	135	67–168
BUN (mg/dl)	45.8	15–60
Creatinine (mg/dl)	1.9	1.0–2.0
Sodium (mmol/l)	151.0	146–157
Potassium (mmol/l)	4.8	3.5–4.8
Chloride (mmol/l)	110	116–126
Total protein (g/dl)	6.08	6.0–8.6
ALT (U/l)	15	<80
AST (U/l)	27	<40
ALP (U/l)	45	<145
GGT (U/l)	6	5.19
Total bilirubin (mg/dl)	0.3	0.1–0.8

- The Clinical Chemistry lab provides **biochemical information** for patient management
- Biochemical tests are performed for most patients in hospitals
- they provide **specific diagnostic information** related to metabolic disorders which give rise to certain disease or those in which biochemical changes are a consequence of diseases.
- The laboratory test usually shows the value of the lab test (referred to the patient result) and the normal reference (range for the normal results)
    - A hemoglobin normal result is 12 with reference range of 10.6-15.6

❖ Biochemical tests are divided into:

1) Qualitative tests

- If certain substance is **present or absent** in the body
    - glucose: should not be present in urine normally as it is not filtered in the glumerulus.
    - Glucosuria (presence of glucose in urine): seen in DM & pregnancy.

2) Quantitative tests

- The quantity or **concentration** of certain substance in the body.
    - glucose concentration in the blood.
    - Glucose conc. of 110-126 mg/dl →impaired fasting glycemia
    - Glucose conc. of >126 mg/dl → DM

❖ **The Analytes**

- **Analyte** is a compound measured in the biochemistry lab whatever this compound is:
- large (albumin) or small (glucose) Mwt.
- organic (amino acids) or inorganic (K^+,Na^+).
- Analytes are basically dissolved in water because most of our bodies consist of water.
- **Concentration** of analyte = **Amount of solute / volume of solvent**.
- so the concentration of analyte depends on two factors:
    - amount of solute
    - volume of solvent
- e.g. glucose concentration differs from fastening state to after eating state.
- Urea:
    - Urea is metabolic waste products of ammonia
    - Its excreted normally with urine
    - In patients with renal failure → the kidney cant excrete it → increase urea amount→ increase in urea concentration
    - In patients with normal kidneys but dehydration → increase concentration because of decrease in solvent (plasma volume)
- It is **not** always when concentration increase or decrease indicate a disease.

- Some analytes are affected by variables related to the patients (age, sex, national status, time…etc).
    - Alkaline phosphatase is higher in children than adults
    - Fasting causes decrease in blood glucose concentration
    - Cortisol levels are higher in morning compared to night
    - Alcohol increases triglycerides
    - Phenytoin causes increase in GGT (gamma-glutamyl enzyme)

Factor	Example of variable affected
Age	Alkaline phosphatase, Urate
Sex	Gonadal steroids
Ethnicity	Creatine kinase
Pregnancy	Urea
Posture	Proteins
Exercise	Creatine kinase
Stress	Prolactin
Nutritional status	Glucose
Time	Cortisol
Drugs	Triglycerides (alcohol) Gamma-glutamyl transferase (phenytoin).

Use of Biochemical investigations:

1) Diagnosis

- Medical diagnosis is based on:
    1. patient's history
    2. physical examination
    3. results of relevant **biochemical investigations**.
        - e.g. Polyurea and polydypsia may indicates DM or diabetes insipidus
        - in case of DM which is due to lack of insulin→ we find elevated blood glucose levels in blood, urine..etc
        - in case of Diabetes insipidus → the levels of ADH hormone is low (in central type)

2) Monitoring

- Follow up the course of illness.
- Follow up the effects of treatment and complications of treatment.
- Follow up disease complications.
- e.g.
    a. Glycated haemoglobin test monitoring in patients with DM.
    b. Use of diuretics requires monitoring of electrolytes to detect hypokalemia, hyperglycemia and increased uric acid.

3) Prognosis:

- Biochemical tests provide information about progression of the disease.
- e.g.
    - Plasma creatinine concentration is indicator for progressive renal disease
        - If plasma creatinine is elevated to certain limit → the patient may need dialysis.
    - Increased plasma cholesterol concentration predicts risk of coronary artery disease.

4) Screening:

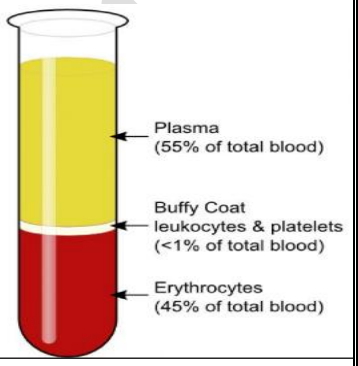
- Biochemical investigations are used to determine whether a condition is present especially in subclinical patients (patients with the disease but yet no symptoms).
- e.g. **Phenylketonuria** (**PKU**):
    - measure serum phenylalanine concentration in newborn babies.
    - Due to deficiency of enzyme called phenylalanine hydroxylase that is responsible for conversion of phenylalanine to tyrosine
    - Inability to convert phenylalanine leads to build up in the blood and eventually **mental retardation**.

❖ The specimen

- The specimen must be appropriate for the test requested.
- The type of the specimen depends on the reason (information) needed for that test.
  - For example, if we want to diagnose DM, we look for blood test
- It is extremely important that the **correct specimen** is collected at the **right time** in to an **appropriate container**.
- Specimens are usually body **fluids:**

1. Blood:

- needs specialized person to collect because the deep arteries are at high blood pressure which increases the risk of bleeding.
  1) **Venous** blood → the most one used
  2) **Arterial** blood → used for special cases → e.g. Gas determination (O2,CO2 pressures)
     a. Needs specialized person to collect because they are deep and increases the risk for bleeding
  3) **Capillary** blood → tip of finger (used for blood typing and in babies)



Test for clotting factors, renin activity → plasma

Electrophoresis → serum

- **Blood sample can be used as (important):**

1. Serum

- Blood without clotting factors.
- Done by Collecting blood without anticoagulant and leaving the sample on the bench for 30 min

2.Plasma

- Blood without cells.
- Blood with anticoagulant → centrifuge → collect the upper part.
  - Anticoagulations used for plasma preparation are;
    - Heparin: prevent coagulation process
    - EDTA/ citrate : chelate Ca^{+2} preventing coagulation factors activation

3.Whole blood

- Blood + anticoagulant.
- Not frequently used.
- E.g. used for blood gases determination.

Serum VS plasma:

- Serum doesn't contain coagulation factors
- Plasma contains coagulation factors

→ If we want to measure the concentration of certain coagulation factor, which test would you choose?
  - Answer: plasma

- Another difference is fibrinogen (coagulation factor) impairs electrophoresis, so we use only serum for electrophoresis not plasma test.
- Another one is Ca^{+2} levels: we use serum not plasma is sometimes because palsma may contain EDTA/citrate which chelate Ca^{+2}

❖ NOTES:

- **Haemolysis** must be avoided when blood is drawn because it increases plasma K^+ & Phosphate conc. & aspartate aminotransferase (AST) activity which come from inside RBCs when they are broken down.
- During **I.V therapy** blood must be drawn from the opposite arm to avoid contamination.
- **Wrong container** leads to wrong results.
  - We can't use bottles containing EDTA, citrate to measure Ca^{+2} levels

- **e.g.** citrate & EDTA (used as anticoagulants in containers) combine with Ca^{+2} leading to low measured concentration in plasma.

❖ **Other types of specimens include:**

1) Urine:

- 24-hours urine collection
  - Used for special cases like creatinine clearance determination
- One sample urine collection
  - Used for microscopial examination for locking for bacteria or crystals (e.g. oxalic acid).

2) Feces:

- Occult blood indicates colon cancer or peptic ulcer.

3) Cerebro-Spinal fluid (CSF)

- Used to diagnose bacterial meningitis

4) Suputm & saliva:

- Used for diagnosis of pneumonia

5) Aspiration of certain fluids:

- Ascites, pleural fluid, synovial (joint) fluid.

❖ **Urgent requests**

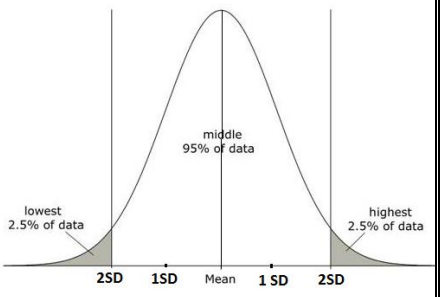
- Examples:
  - Serum [troponin] in a patient with chest pain.
  - Serum [K+] in a patient with Acute renal failure.
  - Serum [paracetamol] in drug overdose.

❖ **Repeat requesting**

- Frequent biochemical investigations should be done when we want to monitor the **progress** of a disease.
- In patients being treated with **thyroxine** for hypothyroidism:
- Normally in patients with hypothyroidism → thyroid stimulating hormone (TSH) levels are elevated
- When treatment with thyroxine starts , it can take several weeks for plasma **[TSH]** to stabilize at a new value after a change in the dose of thyroxine.
- So, repeating thyroid function tests (TSH) in a patient whose dose has been changed at interval of < 1 month may provide misleading information → doctor could change the dose prematurely.
- In contrast, plasma **[glucose] & [K+]** can change very rapidly in patients treated for **diabetic ketoacidosis** → should be monitored every 1-2 hours.

❖ **sample analysis & reporting of results:**

- The ideal analytical method is:
  - **Accurate** = gives a correct result.
  - **Precise** = gives the same result when repeated.
  - **Sensitive** = measures low concentrations of analyte.
    - Meaning can detect very low levels of analyte if existed
  - **Specific** = not subject to interference by other substances.
    - Meaning the test measure only certain analyte not the other (test detect only K+ not Na+)



- In practice**, no test is ideal**, but we must ensure that the results are sufficiently **reliable** to be clinically useful.
- **The Gaussian** distribution of a test values indicates that **there will always be a potential for some degree of** imprecision or **analytical variation** in a result. (Even if the same test is repeated on the same sample).
- It is important to know that results obtained using different methods may **not** be **interchangeable** → to compare between 2 results, same methods should be used in both occasions. يعني ما بقدر اقارن بين تركيز السكر في الدم لمري عمل تحليلين بعتمدوا على طريقتين مختلفتين في التحليل

- It is often appropriate to perform a **group** of **related** tests on a specimen.
- e.g. plasma [Ca], [Phosphate] & alkaline phosphatase activity → all together provide useful information in the diagnosis of bone disease.

❖ **Source of Errors:**

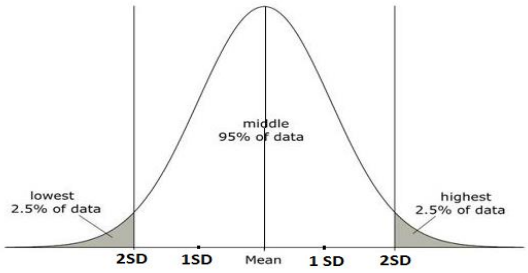
- Errors can occur at various stages of the analysis process:
- **Pre-analytical**
  - wrong specimen collection
  - mislabeling.
  - incorrect preservation.
- **Analytical** → human or instrumental errors occurring in the laboratory.
- **Post-analytical** → incorrect recording in the patient's record (transcription errors).

❖ **Interpretation of results:**

- When the result of a biochemical test is obtained, the following points must be taken into consideration:
  - Is it **normal**?
    - Meaning is it within the reference range
  - Is it **significantly different from previous results**?
    - For example, patient with chronic kidney disease and last month, their creatinine was elevated.
    - If todays test shows normal creatinine levels → this is significantly different from the previous result → could be error
  - Is it **consistent with the clinical findings**?
    - Patient known case of chronic kidney disease must have their creatinine levels elevated

❖ **Reference ranges: (normal values)**

- Normal values versus Reference ranges
- Statistically, reference range refers to a distribution of values from repeated measurements of the same quantity and is described by the bell-shaped **Gaussian curve:**
- The range of the **mean ± 2 standard deviations** represents 95% of the total number of test results →this range represents the **Reference range** of the measured analyte.
- 5% of the test results of the normal population will lie outside the reference interval.



  - This indicate that every laboratory test will have about 5 % of the results of healthy people outside the reference range
  - Meaning not all people test results outside the reference range have an abnormal status (disease)
- Another statistical method is to take sample from 1000 healthy people and run the test → the results are then plotted to give the bell-shaped **Gaussian curve**
- Each laboratory has its own reference ranges which are based on the method of analysis used in the lab.

Q:

- If the mean value for glucose concentration in large number of people using a new test is 100 mg/dl with standard deviation of 10
  - What is the normal reference range using this new test?
    - Ans: 80-100
  - what percentage of healthy people (without DM) may have a test result above 100 and still considered not to have DM?
    - ans: 2.5