Biochemistry Practical

Biochemistry Teamwork

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Biochemistry practical "Estimation of blood glucose in DM".

(Dr.Sumbul said the most important thing you need to know is the test for DM. The calculations are not the important but they may come)

Inrtoduction:

- Blood glucose is normally maintained within a narrow range under various conditions by insulin, glucagon and other hormones
- The most common disorder of carbohydrate metabolism is diabetes mellitus characterized by high blood glucose level
- Measurement of blood glucose is one of the most commonly performed tests in clinical biochemistry labs of hospitals
- Signs and symptoms of DM: Hyperglycemia, Polyuria and glucosuria, Polydipsia and Polyphagia.

Comparison between type 1 and type 2 DM

	Type 1 Diabetes	Type 2 Diabetes
Age of onset	Usually during childhood or puberty	Frequently after age 35
Prevalence %	10% of diagnosed diabetics	90% of diagnosed diabetics
Defect or deficiency	β cells are destroyed, eliminating insulin production	Insulin resistance combined with inability of β cells to produce appropriate quantities of insulin
Ketoacidosis	Common	rare
Plasma Insulin	Low to absent	High early in disease; low in disease of long duration
Treatment	Insulin is always necessary	Diet, exercise, oral hypoglycemic drugs, +/- insulin

Laboratory tests for glucose (V.imp)

1) Fasting plasma glucose (FPG): is measurement of plasma glucose after 12 hours of fasting (no caloric intake)

Normal level: 3.9-5.6 mmol/l (70-100 mg/dL).

2) OGTT (Oral Glucose tolerance Test) and 2-hour post-prandial test:

Serial measurement of plasma glucose before and after a specific amount of glucose given orally (75g glucose)

3) HEMOGLOBIN A_{1C} :

** It is produced due to non-enzymatic glycosylation of hemoglobin.

** It is used to estimate glycemic control in the last 1-2 months

**** Recommended for the detection of type 2 DM**

** HBA_{1C} and fasting plasma glucose are effective in diagnosing diabetes

**** Cut-off point of ≥ 6.5 % is used to diagnose diabetes**

NOTE: When fasting plasma glucose or 2-hour postprandial glucose level is above normal but below diabetic level ⟶ **impaired glucose tolerance**

Categories of increased risk for diabetes*

FPG 100-125 mg/dL (5.6-6.9 mmol/L) [IFG]
2-h PG on the 75-g OGTT 140-199 mg/dL (7.8-11.0 mmol/L) [IGT]
A1C 5.7-6.4 percent

FPG: fasting plasma glucose; IFG: impaired fasting glucose; PG: post glucose; OGTT: oral glucose tolerance test; IGT: impaired glucose tolerance; A1C: glycated hemoglobin.

Criteria for the diagnosis of diabetes

1. A1C ≥6.5 percent. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* **OR**
2. FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.* **OR**
3. Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.* **OR**
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

A1C: glycated hemoglobin; NGSP: National glycohemoglobin standardization program; DCCT: Diabetes control and complications trial; FPG: fasting plasma glucose; OGTT: oral glucose tolerance test.
* In the absence of unequivocal hyperglycemia, criteria 1-3 should be confirmed by repeat testing.

Diagnosis	Mg/dL	Mmol/L
Normal	**Below 100**	**Below 5.6**
Impaired Glucose Tolerance	**100 - 125**	**5.6 – 6.9**
Diabetes Mellitus	**FPG more than 126** **OGGT more than 200** **Rondom test more than 200**	**FPG more than 7.0** **OGTT more than 11.1** **Random test more than 11.1**

- ***You maybe asked about the interperation of the result or the diagnosis.***
- ***IF you answers a question in (mmol/L), you should continue with the same measuring unite***
- ***You are free to choose between (mg/dL) & (mmol/L).***

Blood glucose Assay: (The doctor said we will not ask you anything about the procedure; just know the equation in case they ask you to calculate.)

*Blood glucose is detected by a series of enzymatic reactions that ultimately form a colored product.

*The intensity of color is proportional to the amount of glucose present in blood

*Color intensity is determined spectrophotometerically by measuring the absorbance of the colored solution at a wavelength of 546nm

Calculation

Glucose conc (mmol/l) = (Normal >>> 3.9–5.6 mmol/L (70–100 mg/dL)

$$\frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc. of standard (5.6 mmol/l)}$$

Ketone Bodies:

- Acetone (exhaled by lungs, gives characteristic smell in diabetic ketoacidotic patients)
- Acetoacetate
- β-Hydroxybutyrate
- Produced by the liver and utilized for energy production by peripheral tissues

Ketone bodies are detected in urine using >>> Urine dipstick.

Urinalysis using dipstick: (Not imp)

Principle:

**Dipsticks are plastic strips impregnated with chemical reagents which react with specific substances in the urine to produce color-coded visual results.
**They provide quick determination of pH, protein, glucose and ketones. The depth of color produced is proportional to the conc. of the substance in urine.
**Color controls are provided against which the actual color produced by the urine sample can be compared.

Procedure:

- Dip the dipstick in the urine sample provided
- Remove it immediately
- Wipe off excess urine
- Read the color produced within 60 seconds
- Compare color changes with the control charts provided