

Subject; Chemical Pathology
Lectures for Ameer-Ud-Din Medical
College 4th Year MBBS.

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Chemical Pathology.

Lecture ; 1 Topic ; **Estimation of serum Glucose by GOD/POD Method.**

INTRODUCTION

1. Carbohydrate metabolism produces glucose which is the primary energy source of the body. The concentration of the glucose is kept in a fairly narrow range in the blood via complex interplay of various path ways modulated by a number of hormones. Insulin is the anabolic hormone which facilitates the glucose entry into cells therefore reduces blood glucose level and the other counter regulatory hormones like glucagon , epinephrine , cortisol , growth hormone, which increases the blood glucose level.
2. The most frequently encountered disorder of carbohydrate metabolism is hyper glycemia, which is caused by diabetes mellitus.
3. Patient with diabetes mellitus demonstrates inability to produce or utilize insulin.

Elaboration & Explaining the Methods

GOD (Glucose oxidase) – POD Peroxidase Method.

Principal;

D – Glucose + 2H₂O + O₂  Gluconic Acid + H₂O₂. (GOD)

2H₂O₂ + Phenol + 4amino anti pyrin  quinone + 4H₂O. (POD)

Reference Range;

Serum Fasting glucose = 60 – 100 Mg / Dl.

Serum random glucose = 60 – 140 Mg / Dl.

Materials Required for Method

1. Cuvettes and test tubes.
2. Reagent , standard and specimen.
3. Spectrophotometer.

Specimen required;

- I. Serum.
- II. Plasma.
- III. hyphenized serum.

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Reagents required;

- I. Phosphate buffer.
- II. Phenol.
- III. GOD , POD.
- IV. 4 Amino anti-pyrins.

Assay Conditions;

- 1- Wave length at 546 nm .
- 2- Temperature @ 37Degree Centigrade
- 3- Cuvettes 1 Centimeter Light Path.
- 4- Zero Adjustment against reagent blank.

PROCEDURE

- 1- Start the spectrophotometer.
- 2- Adjust Wavelength at 546 NM.
- 3- Take 3 test tubes and label them as blank , standard and test.
- 4- Add 1 ml of working reagent in all test tubes.
- 5- Add 10 Micro Litre of standard solution in test tube labelled as standard(S).
- 6- Add 10 Micro Litre of specimen in test tube labelled as test (T).
- 7- Mix all test tubes and incubate them at 37 Degree Centigrade and read the absorbance against reagent blank.

Table;

Sr.#		Blank LB	Standard (S)	Specimen (T).
1-	Working Reagent	1 ml	1 ml	1 ml
2-	Standard	-	10ul	-
3-	Specimen	-	-	10 ul

Observation & Calculation;

Serum Glucose Concentration = Absorbance of specimen / absorbance of standard * Concentration of standard

Clinical Interpretations;

Causes of hyper glycemia.

1. Diabetes Miletus.
2. Medical Problem such as stroke, heart attack , pancreatitis.
3. Trauma such as Burn or injury.
4. Infections such as pneumonia or UTI.
5. Medicine such as steroids or diuretics and norcotics e.g cocaine and ecstasy.

CONTINUE.....

6. Surgery.
7. Polycystic ovarian syndrome.
8. History of gestational DM.
9. Family History of Diabetes.
10. Obesity or a sedentary lifestyle.

CAUSES OF HYPOGLYCEMIA

1. Over doze of insulin.
2. Over consumption of Alcohol.

CONTINUE.....

3. Insulinomas.
4. Drugs (Salicylates, Sulpha Drugs).
5. Low Level of cortisol , Growth Harmon , glucagon , epinephrine.

NORMAL VALUES & INTERPRETATIONS.

Lab Findings in Hyper glycemia;

1. ↑ Increased Blood glucose level.
2. ↑ Increased Urine Specific gravity.
3. ↑ Increased Serum and urine osmolality.

CONTINUE.....

4. Ketones in serum and urine.
5. Decreased blood and urine PH.
6. Electrolyte imbalance.

Clinical Interpretations;

Normal Fasting Glucose = 70 -99 mg/dl.

Impaired fasting glucose = 100-125 mg/dl.

Provisional diabetes > 126 mg/dl.

DIGNOSTIC CRETERIA FOR DIABETES.

1- FBS > 126 mg/ dl.

2- RBS > 200 mg/dl.

3- 2 Hour PLASMA GLUCOSE > 200 mg/dl.

4- HBA1C > 6.5 %

CRITERIA FOR TESTING FOR PRE-DIABETES & DIABETES;

All Adults above 45 Years of age should be tested every three years using either HBA1C or FBS. If BMI is more than 25kg / meter m square testing should be carried out at an earlier age or more frequently. Additional risk factors are; 1 Physically inactive . 2. Family History of Diabetes . 3. High risk minority population (e.g African Americans , Latinos , Native Americans & Pacific Islanders.

CRITERIA FOR TESTING FOR PRE-DIABETES & DIABETES;

Continue.....

4. History of gestational DM or delivering a baby weighing more than 4.1 kg.
5. Hypertension.
6. Dyslipidaemia.
7. History of Polycystic Ovarian Syndrome.
8. History of CVS diseases.

CRITERIA FOR TESTING FOR TYPE 2 DM IN ASYMPTOMATIC CHILDREN.

This criteria include initiation of testing at the age of 10 Years or at the on set of puberty with follow-up testing every two years. Testing should be carried out on the children who display the following characteristics. 1- Over wight children . 2- Family History in first & second degree relative or 3. Race e.g Native American , African American , Latinos.

DIAGNOSTIC CRITERIA FOR GESTATIONAL DM

- 1- Fasting plasma glucose > 92 mg/ dl .
2. 1 Hour Plasma Glucose > 180 mg /dl.
3. 2 Hours Plasma Glucose > 153 mg/dl.

Most glucose measurements are performed on serum or plasma. The glucose concentration in whole blood is approximately 11% Lower than the glucose concentration in plasma. Serum or plasma must be refrigerated and separated from cell within one hour to prevent the substantial loss of glucose by the cells. Particularly if the WBC count is elevated. Sodium Fluoride (Gray top tube) is often used as anti coagulant and preservative of whole blood particularly if analysis is delayed. The fluoride inhabits glycolytic enzymes. Therefore the plasma should be separated as soon as possible. Fasting blood glucose should be obtained in the morning after an approximately 8 to 10 hours fasting (No longer than 16 Hours).

DIAGNOSTIC CRITERIA FOR GESTATIONAL DM

Fasting plasma glucose have a diurnal variation. The mean FBG higher in the morning than in the afternoon.

Renal Glucosuria.

Normal threshold for glucose is 170/180 mg/dl. If blood sugar rises above this level glucose starts to appear in urine this is called glucosuria.

GLUCOSE TOLERANCE AND 2 HOURS POST PRANDIAL TESTS.

Preparation for oral glucose tolerance test include;

- 1- On normal to high carb diet for three days.
- 2- Should be fasting for 10 hours but not more than 16 hours.
3. Should be performed in morning to avoid diurnal effects.
4. Should be refrain from exercise before and during testing.

GLUCOSE TOLERANCE & 2 HOURS POST PRANDIAL TESTS.

CONTINUE.....

5. Should be refrain from eating , drinking (Except water) and smoking before and during testing.
6. OGTT is effected by 1- Large doses of salicylates. 2- Diuretics. 3. Anti-Convulsants 4. Oral contraceptives and steroids.
7. OGTT is not recommended for routine use according to ADA guidelines.
8. Indications for OGTT includes 1. family history of diabetes 2. previous history of large babies. 3. When there is impaired fasting glucose.

This procedure is inconvenient to patients and it is not used by physicians for diagnoses of DM. In OGTT 75gm of glucose for adults and 1.75 gms /kg for children is given orally in form of solution and we take blood samples after 2 hours for estimation of blood glucose. In OGTT we take fasting blood sample than glucose solution is given and than we take sample after 2 hours for estimation of blood glucose.

CONTINUE.....

If patient is pregnant lady than we take an additional blood sample at the end of 1 hour.

GLYCOSYLATED HEMOGLOBIN (HBA1C).

It is the term use to describe the formation of a hemoglobin compound produced when glucose reacts with amino acids group of hemoglobin. Average red blood cells lives approximately 120 days so the glycosylated HB level at any one time reflects the average blood glucose level over the period of two to three months.

Glucose molecule attached to one or both N terminal valines of the Beta Poli peptide chains of normal adult HB.

Normal values of HBA1C range from 4% to 6%.

According to ADA guidelines HBA1C test be performed at least two times a year with patient who are meeting treatment goals and who have stable glycaemic control.

CONTINUE

For patient whose therapy has changed or who are not meeting glycaemic goals a quarterly HBA1C test is recommended.

Specimen requirement for HBA1C Measurement is an EDTA Whole blood sample. (No fasting is required and test can be performed at any time).

In Lab HPLC (High performance liquid chromatography) is used for estimation of HBA1C.

That's all Students.

THANKS WILL BE BACK WITH ANOTHER LECTURE.