

## Investigations used in Diabetes Mellitus

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## Commonly performed tests

- Glucose
  - FPG
  - PPBS
  - OGTT
  - Capillary blood glucose
- HBA1c
- Serum Fructosamine
- Urine microalbumin
- Ketones
- Lipid profile
- RFT
- LFT

## Diagnosis with plasma glucose

- Thirst, polyuria and weight loss
    - Fatigue, lack of energy, blurring of vision or recurrent infections
  - Symptomatic/Asymptomatic
  - fasting glucose of  $\geq 126$  mg/dL
- or
- a random glucose of  $\geq 200$  mg/dL

## Fasting plasma glucose

- Fasting morning venous glucose is the best initial test for diagnosing diabetes.
- 8-12 hours fasting (10)
- The FPG test has been the most common test used for diagnosing diabetes because it is more convenient than the OGTT and less expensive.
- The FPG test measures blood glucose in a person who has fasted for at least 8 hours and is most reliable when given in the morning.

## OGTT

- When the results of fasting or random blood glucose are equivocal
  - Fasting 100-126 mg/dL
- Unnecessary if fasting or random blood glucose values are clearly in the nondiabetic or diabetic range

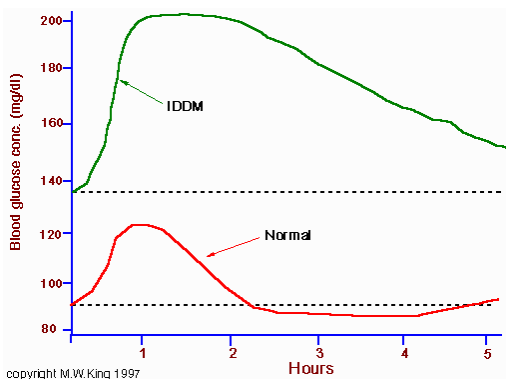
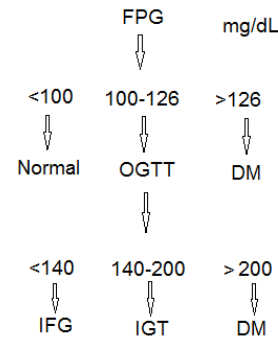
## Preparation for OGTT

- Unrestricted carbohydrate diet for 3 days prior to the test.
- Fasting for at least **8 hours**.
- Water allowed.
- Morning medication is omitted and taken when test completed.

## OGTT procedure

- Fasting blood sample collected.
- 75g anhydrous glucose dissolved in 300 mL of water.
- Time of patient finishing the glucose load is recorded
- Second blood sample is taken at 2 hours.
- Patient should be seated & remain within the department throughout test.
- If any glucose is lost by vomiting, test should be discontinued.

## OGTT results



## Capillary blood glucose

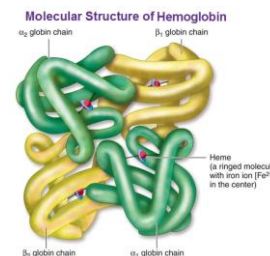
- Another way of testing glucose conc. In blood.
- A small drop of blood is placed on a disposable test strip which interfaces with a digital meter.
- Within several seconds, the level of blood glucose will be shown on the digital display.
- Patients are advised on the appropriate monitoring regime for their condition.

## Capillary blood glucose cont.

- Finger-stick devices, are not accurate enough for diagnosis but may be used as a quick indicator of high blood glucose.



## HBA1<sub>c</sub>



Glucose (covalently linked) to the N-terminal valine of the  $\beta$  chain.

## HbA1c biochemistry

- A molecule of hemoglobin is made up of 4 protein chains.
- There are 2 alpha chains and 2 beta chains, usually denoted as  $\alpha_2\beta_2$ .
- Glucose will react and bond to certain chemical groups on the hemoglobin.
- These are located at the start (N-terminal end) of each of the  $\alpha$  and  $\beta$  chains and on some amino acid side chains within the protein.

## HbA1c biochemistry

- Glycated hemoglobin is defined as hemoglobin with glucose bound to any of these potential sites.
- HbA1c is a subset of glycated hemoglobins.
- It is defined as hemoglobin with glucose bound at the beginning (N-terminal) of the  $\beta$ -chain.
- The total glycated hemoglobin will include HbA1c plus all the other hemoglobins that have glucose bound to lysine side chains and/or to the N-terminal of the  $\alpha$ -chain.

## What affects the HbA1c level

1. The concentration of glucose that the haemoglobin is exposed to
  - if glucose levels in the plasma is high, more glycosylation of haemoglobin will occur.

## What affects the HbA1c level

2. The length of time that the haemoglobin is exposed to a given concentration of glucose.
  - life span of a red blood cell (120 days).
  - Haemolytic anaemia-shortened rbc life span-↓ GHB
  - Recent blood loss-more new rbc-↓ GHB
  - Iron deficiency anaemia-more older rbc-↑ GHB

## Glucose vs HbA1c

- Sugar levels fluctuate from minute to minute, hour to hour, and day to day.
- Thus for hour to hour control, or day to day, a sugar level is the best guide.
- The HbA1c level changes slowly, over 8 weeks, so it can be used as a 'monitoring' test.

## HbA1c for diagnosis of DM

- It can be performed at any time of the day and does not require any special preparation such as fasting.
- These properties have made it the preferred test for assessing glycaemic control in people with diabetes.
- HbA1c has now been recommended as a means to diagnose diabetes.

## HbA1c for diagnosis of DM

- Diagnosis can be made if the HbA1c level is  $\geq 6.5\%$ .
- Diagnosis should be confirmed with a repeat HbA1c test, unless clinical symptoms and plasma glucose levels  $>126/200$  mg/dl are present.

## Limitation of HbA1c

- Situations where the average RBC lifespan is altered;
  - Blood loss
  - Haemolytic anaemia
  - Iron deficiency anaemia
- Haemoglobinopathies
  - HB S
  - HB C

## Fructosamine

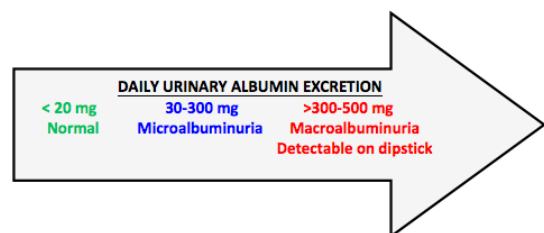
- Glucose attached to other plasma proteins.
- Glycated serum albumin.
- Half life of serum albumin is 20 days.
- This measures short term control of blood sugar for the past 2-3 weeks.

## Fructosamine

- Useful in
  - Haemolytic anaemia
  - HB variants (HB S, HB C)
  - Pregnancy
- This measures short term control of blood sugar for the past 2-3 weeks.

## Urine microalbumin

- The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels of albumin in the urine.
- Once overt nephropathy occurs, without specific interventions, the glomerular filtration rate (GFR) gradually falls over a period of several years.
- Once clinical proteinuria occurs ( $>500$  mg/L) progressive renal damage is likely.



## Sample collection

- 24 hour urine collection (gold standard method)
- albumin:creatinine ratio (ACR)—in a first morning void or in a spot urine sample (commonly done method)

## Sample collection cont.

- Recent exercise (within 24 hours before the test)
- Recent sexual intercourse (within 24 hours before the test) in males
- Illness
- UTI
- Menstruation or pregnancy in women

## Ketones

- Ketones are produced when there is a shortage of insulin in the blood and the body breaks down body fat for energy.
- Ketones in the urine is a sign that your body is using fat for energy instead of using glucose because not enough insulin is available to use glucose for energy.
- When levels get too high, you can develop DKA.

## Ketones cont.

- There are three ketone bodies;
  - Acetoacetate
  - Acetone
  - beta-hydroxybutyrate
- Blood testing gives a snapshot of the status of ketone accumulation at the time that the sample was collected.
- Rothera's test

## Ketones cont.

- The urine methods measure either acetoacetate or acetoacetate and acetone but do not detect beta-hydroxybutyrate.
- The major ketone produced in DKA is beta-hydroxybutyrate
- Because the assay only detects acetoacetate, the initial ketoacidosis may be more severe than indicated by the urine ketone level.

## Ketones cont.

- After treatment beta-hydroxybutyrate is converted to acetoacetate.
- Thus, the ketoacidosis may appear to worsen with treatment and urine ketones may remain detectable long after recovery of the acid-base status.
- Blood beta-hydroxybutyrate and/or acid-base status may be a better indicator for managing DKA.

## Lipid Profile

- Lipid abnormalities are common in diabetics and frequently seen in type 2 diabetics.
- Dyslipidaemias make diabetics prone to develop CHD and other complications of atherosclerosis.

## Lipid Profile cont.

- High plasma triglyceride
- Low HDL cholesterol concentration
- Increased level of LDL cholesterol

## Other tests

- Renal function tests
  - BU
  - S Creatinine
  - GFR
- Liver function tests
  - SGOT
  - SGPT
  - Gamma GT
  - ALP