

## INVESTIGATIONS IN DIABETES MELLITUS

### 1. Routine investigations

CBC

### 2. Laboratory tests for diagnosis of diabetes

The tests for the diagnosis of diabetes are given in Table 1.

**Table 1**  
**Laboratory tests for the diagnosis of diabetes**

Fasting Plasma glucose 2 hours plasma glucose Random Plasma glucose OGTT HbA <sub>1C</sub>
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#### a. Fasting plasma glucose

- The fasting plasma glucose (FPG) test (fasting blood sugar test), is a simple, noninvasive blood test
- Prior to being tested, a person must be fasting for 12 to 14 hours. Because of this, the test is usually done in the morning

#### Interpretation

- *Normal* – FPG between 70 mg/dL to 99 mg/dL
- *Pre-diabetes – Impaired fasting glucose* - – FPG between 100 mg/dL to 126 mg/dL
- *Diabetes* - A reading above 126 mg/dL is the threshold at which diabetes is diagnosed.

#### b. OGTT

In an *oral glucose tolerance test* glucose is administered by mouth. How quickly the glucose is cleared from the blood and homeostasis is maintained is determined by measuring serial plasma glucose levels.

- The WHO recommendation is for a 75g oral dose in all adults: the dose is adjusted for weight only in children. The glucose dose is taken within 5 minutes.
- Blood is drawn at intervals for measurement of glucose (blood sugar). For simple diabetes screening, the most important sample is the 2 hour sample and the 0 and 2 hour samples may be the only ones collected.

#### Interpretation (Table 2)

- **Fasting plasma glucose** (measured before the OGTT begins) is normally below 100 mg/dl. (6.1 mmol/l)
  - Fasting levels between 100 and 125 mg/dl (6.1 to 7.0 mmol/l) are borderline ("impaired fasting glycaemia")
  - Fasting levels repeatedly at or above **126 mg/dl** (7.0 mmol/l) are diagnostic of diabetes

- The **2 hour OGTT** glucose level should be below 140 mg/dl (7.8 mmol/l).
  - Levels between 140-200 mg/dl ( 7.8 mmol/l to 11.1 mmol/l) indicate "impaired glucose tolerance"
  - Glucose levels above **200** mg/dl (11.1 mmol/l) at 2 hours confirms a diagnosis of diabetes

**Table 2**  
**1999 WHO Diabetes criteria - Interpretation of Oral Glucose Tolerance Test**

Glucose levels	NORMAL		impaired fasting glycaemia (IFG)		impaired glucose tolerance (IGT)		Diabetes Mellitus (DM)	
	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs
(mmol/l)	<6.1	<7.8	> 6.1 & <7.0	<7.8	<7.0	>7.8	>7.0	>11.1
(mg/dl)	<100	<140	>100 & <126	<140	<126	>140	>126	>200

c. *HbA<sub>1c</sub> (Glycated hemoglobin)*

- When hemoglobin is exposed to high plasma levels of glucose, glycated hemoglobin is formed in a non-enzymatic pathway
- Glycation of hemoglobin has been associated with cardiovascular disease, nephropathy and retinopathy in diabetes mellitus
- HbA<sub>1c</sub> is used primarily to identify the average plasma glucose concentration over prolonged periods of time
- Currently HbA<sub>1c</sub> has been included as a test for diagnosis of diabetes.
- Monitoring the HbA<sub>1c</sub> in type-1 diabetic patients may improve treatment.

HbA<sub>1c</sub> is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management. HbA<sub>1c</sub> assays are now highly standardized so that their results can be uniformly applied both temporally and across populations. In their recent report, an International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the HbA<sub>1c</sub> test to diagnose diabetes, with a threshold of  $\geq 6.5\%$ ,

*Principle of HbA<sub>1c</sub> test*

The normal life span of the RBCs is about 120 days. During this period, glucose molecules react with hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, the quantities of these glycated hemoglobins are much higher than in healthy people.

Glycation of hemoglobin is an irreversible process. A rise of glycated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring

long-term serum glucose regulation. The HbA<sub>1c</sub> level is proportional to average blood glucose concentration over the previous four weeks to three months.

The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the HbA<sub>1c</sub>  $\geq$  6.5% as other criteria for the diagnosis of diabetes.

### Criteria for diagnosis of diabetes

**Table 4**  
**Criteria for diagnosis of diabetes (ADA)**

<p>1. <b>HbA<sub>1c</sub> <math>\geq</math> 6.5%. *</b> OR</p> <p>2. <b>FPG <math>\geq</math> 126 mg/dl (7.0 mmol/l). *</b> Fasting is defined as no caloric intake for at least 8 hours OR</p> <p>3. <b>2-h plasma glucose <math>\geq</math> 200 mg/dl (11.1 mmol/l) during an OGTT*.</b> 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</p> <p>4. <b>In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose <math>\geq</math> 200 mg/dl (11.1 mmol/l).</b></p> <p><i>*In the absence of unequivocal hyperglycemia criteria 1–3 should be confirmed by repeat testing performed every year.</i></p>
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### 3. Tests for diagnosis of Type 1 diabetes

#### a. Insulin

This test is used for determination of concentration of bioavailable insulin in the patients. Total insulin exists in free and bound form. In patients without insulin antibodies, total and free levels are similar, but in patients with insulin antibodies total insulin levels are dependant on the binding capacity of the circulating endogenous insulin antibody and availability of insulin to bind to antibody sites. This test is used to determine dosage of IDDM with insulin antibodies. Free insulin measurement helps in interpreting blood sugar concentration and its relationship to insulin injections in insulin treated pts with insulin antibodies. Elevated blood glucose with low free insulin level indicates insufficient insulin for adequate control. Low blood glucose with high free insulin level indicates the need to change the dosage

#### b. Insulin antibodies

Most common antibodies are IgG, IgM, IgA & IgE Abs have been reported. These antibodies are generally seen in pre-Type I DM as well as DM pts with exogenous bovine

or human porcine insulin. Widespread use of human insulin & improved purity of animal insulin has led to significant decrease in insulin antibodies.

### **c. Free Insulin**

Increased levels of free insulin are seen in exogenous insulin, insulinoma, insulin resistance and Type II DM.

### **d. Proinsulin**

Proinsulin is produced in beta cells of pancreas and cleaved into insulin and C-peptide before release into circulation. Only 2-3 percent of proinsulin escapes the conversion and is secreted into blood. Proinsulin is produced in beta cells of pancreas and cleaved into insulin and C-peptide before release into circulation. Increased levels are seen in insulinomas, severe hypoglycemic hypoinsulinoma and hyperproinsulinemia.

Proinsulin inhibits hepatic production of glucose thus useful in type II DM. TG & HDL concentrations improve with proinsulin. It is used as agonist with insulin due to longer elimination time  $\frac{1}{2}$  life and lower metabolic rate. Thus Proinsulin serves as analogue to insulin to retard the complications of Type II DM.

### **e. GAD Antibodies**

GAD-65 Antibodies: GAD is known as Glutamic Acid Decarboxylase. They are detected in approximately 90 per cent of patients who are newly diagnosed of Type I DM and 80 per cent of pre-diabetic individuals and first degree relative of patients with IDDM.

### **f. C-Peptide**

C-peptide is cleaved from proinsulin and released into circulation in the course of insulin biosynthesis. C-peptide is used for assessment of pancreatic islet cell function. Type II DM is associated with abundant C-peptide secretion whereas Type I DM has little or no C-peptide.

## **4. Tests for detection of complications**

- a. Urine routine, urine microalbuminuria, BUN, Serum Creatinine for detection of nephropathy

- Microalbuminuria is the first warning signal to an impending “Nephropathy”. Patients with microalbuminuria have a greater risk for developing renal failure, vascular damage and risk for cardiovascular damage. It can be estimated by immunoturbidometry and nephelometry
- b. ECG, Echocardiography, Stress test for evaluation of cardiovascular complications
  - c. Duplex ultrasound , magnetic resonance angiogram, angiography of lower limbs for evaluation of PAD
  - d. Biothesiometry, Nerve conduction studies, EMG for evaluation of neuropathy
  - e. Fundoscopy, FFA, OCT for evaluation of retinopathy/maculopathy

### Brain Teaser

1. For a diagnosis of diabetes, the fasting plasma glucose should be
  - a) Above 200 mg %
  - b) Above 140 mg%
  - c) Above 126 mg%
  - d) Above 110 mg %
  
2. For a diagnosis of diabetes, the 2 hour plasma glucose should be
  - a) Above 200 mg %
  - b) Above 140 mg%
  - c) Above 126 mg%
  - d) Above 110 mg %
  
3. In a patient with symptoms of hyperglycemia, for a diagnosis of diabetes, the random plasma glucose should be
  - a) Above 200 mg %
  - b) Above 140 mg%
  - c) Above 126 mg%
  - d) Above 110 mg
  
4. For a diagnosis of diabetes, the HbA1C value should be
  - a) Between 5 to 5.6%
  - b) Between 5.7 to 6.4%
  - c) Above 6.5 %
  - d) Neither of the above
  
5. Categories for increased risk of diabetes include
  - a) Impaired fasting glucose
  - b) Impaired glucose tolerance
  - c) HbA1C between 5.7 to 6.4
  - d) All of the above

### Answers

- 1 c, 2 a, 3 a, 4 b, 5 d.

