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Review Article

Diabetes Mellitus and its Laboratory Diagnosis

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Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia either due to deficiency of insulin production or resistance of organs to the effect of normal amount of insulin or both.[1] In healthy individuals after taking meals, according to the blood glucose concentration appropriate amount of insulin is produced by the pancreas and this insulin transports glucose from blood into the cells. In diabetic individual, either little or no insulin is produced by the pancreas, or the cells do not give appropriate response to the produced insulin. This insulin dysfunction leads to decreased synthesis and increased degradation of glycogen, protein and fat in the body, ultimately causing hyperglycemia and overflow of glucose into urine.[2] Characteristic symptoms of diabetes are thirst, polyuria, weight loss and blurring of vision. Most severe form is characterized by ketoacidosis or non ketotic hyperosmolar coma, ultimately leading to stupor, coma and death. Chronic hyperglycemia develops pathological and functional changes characterized by retinopathy, nephropathy and neuropathy. Risk of cardiovascular, peripheral vascular and cerebrovascular diseases is also common in chronic state.[3]

Diabetes is a universal health issue. Its prevalence is continuously increasing worldwide. Estimated rise in world prevalence of diabetes is 6.4% to 7.7% from 2010 to 2030. Different factors like population growth, ageing and sedentary life styles are responsible for continuously increasing burden of diabetes in developing countries. In Pakistan in 2010 about 7.1 million people were suffering with diabetes. This number is expected to rise up to 13.8 million by 2030.[4] On the basis of etiology diabetes can be classified into various types (Table 1). Among all of them most important are type I, type II and gestational diabetes mellitus.

Type 1 diabetes

Type 1 diabetes was previously known as insulin dependent diabetes or juvenile onset diabetes. Being an autoimmune disease, it is characterized by destruction of insulin producing beta cells in the pancreas by the body's own immune system. In turn insulin production is either decreased or completely lost depending upon the extent of pancreatic injury.[5] Type 1 diabetes, although most commonly encountered during childhood and adolescence, can also be diagnosed as late as the 8th or 9th decade of life.[6] Rate of destruction of pancreatic beta cells varies from very high to low among different age groups. Most frequently in children, but also in some adults, rapid progressive beta cells destruction is commonly manifested by absolute insulin deficiency. On the other hand, a slowly progressive form is usually observed in adults and is sometimes known as "latent autoimmune diabetes in adults (LADA)".[7,8]

Genetic inclination and environmental factors play an important role in its pathogenesis.[5] Some individuals who present with permanent hypoinsulinemia, have tendency to develop ketoacidosis, but have no evidence of autoimmunity, are labeled as type 1 idiopathic diabetes.[9] In individuals with type 1 diabetes process of beta cells destruction usually starts very early, but symptoms may appear late. That is why in 85-90% of such individuals when first diagnosed with fasting hyperglycemia, markers of immune destruction including islet cell autoantibodies and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) will also be detected in their blood.[10] Role of HLA, with linkage to DQA and DQB genes and influence by DRB genes is also evident in type 1 diabetes.[5] Children usually present with severe symptoms, marked hyperglycemia or ketoacidosis as the first manifestation of the disease.[11] While some individuals present with moderate hyperglycemia and in the presence of different contributory factors like infection or stress this moderate form may deteriorate into severe hyperglycemia or ketoacidosis. Adults having type 1 diabetes usually present with less severe symptoms due to residual beta cell functions. These individuals develop ketoacidosis after many years of diagnosis when beta cells completely lose their function leading to little or no insulin production as manifested by low or undetectable levels of plasma C-peptide.[7,12] Patients with type 1 diabetes may also suffer from other autoimmune disorders like Grave's disease, Hashimoto's thyroiditis and Addison's disease.[13]

Type 2 diabetes

Type 2 diabetes was previously recognized as non insulin dependent diabetes or adult onset diabetes. It is the most common form of diabetes characterized by relative insulin deficiency. In individuals presenting with type 2 diabetes, at the time of diagnosis the production of insulin from

pancreas is sufficient, but ability of the body to utilize this insulin decreases; the condition is called insulin resistance that leads to hyperglycemia. Over the subsequent years, the function of beta cells also decreases gradually; concentration of glucose in blood further enhances and body is no more able to do efficient use of its main source of fuel.[14,15] Initially hyperglycemia is not so severe as to produce considerable symptoms; therefore, the patients are usually diagnosed after many years. Long term risk of micro- and macrovascular complications is greater in such patients.[16] Ketoacidosis rarely occurs in this type of diabetes. If present it usually occurs in the presence of infection or some other illness.[5]

This form of diabetes is most often associated with older age, obesity and other risk factors (Table 2). About 80 percent of people with type 2 diabetes are overweight or obese.[17] Obesity itself is responsible for insulin resistance in type 2 diabetes. Due to the polygenic nature, type 2 diabetes may involve polymorphisms in multiple genes that encode the proteins involved in insulin signaling, insulin secretion and intermediary metabolism.[2]

Gestational diabetes

Gestational diabetes is defined as carbohydrate intolerance with onset or first recognition during pregnancy (approximately after 24 weeks of gestation). In pregnancy, up to first half of second trimester glucose levels, both in fasting and postprandial states are usually lower than such levels in normal non pregnant women. Women having increased glucose concentration during this time are usually labelled as having overt diabetes mellitus, but not the gestational diabetes. Risk of gestational diabetes in women increases with increasing age, in those who have previous history of glucose intolerance, and those who have history of 'large for gestational age babies', in different ethnic groups. Women having these risk factors should be screened during the first trimester in order to rule out the previously undiagnosed diabetes mellitus. Gestational diabetes occurs due to placental hormone changes that affect the insulin functions. Gestational diabetes mellitus usually disappears after the birth of baby, but there are greater chances of developing diabetes mellitus type 2 within next few years. Maintenance of body weight and appropriate physical activity may help in reducing this risk.[3,17]

Other specific types

Other specific types of diabetes, which are relatively less common aredepicted in table 1.[5]

Pre diabetes

Pre diabetes is defined as a condition in which the blood glucose concentration is greater than normal but lesser than the levels essential for the diagnosis of diabetes mellitus. This encompasses the conditions of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) representing glucose regulation abnormalities during fasting and post-prandial state, respectively. Due to insulin resistance, pancreatic beta cells initially undergo compensatory state and produce larger amount of insulin than normal. This higher concentration of insulin tries to overcome the increase demand and ultimately maintains the blood glucose concentration some way intermediate between normal glucose levels and diabetic glucose level.[3,17]

Hemoglobin A1C

Hemoglobin A1C (HbA1C) is stable glycosylated hemoglobin. It shows the average blood glucose level of last 2-3 months (120 days life span of erythrocytes). It gives a percentage that indicates the risk of development, diagnosis and previous 2-3 months control of diabetes. In certain conditions HbA1C is not considered appropriate for diagnosis of diabetes. These conditions include[18]

1. ALL children and young people
2. Patients of any age suspected of having Type 1 diabetes or type 2 diabetes with a severe insulin deficiency (glycemic variability)
3. Patients with symptoms of diabetes for less than 2 months
4. Patients at high diabetes risk who are acutely ill (e.g. those requiring hospital admission)
5. Patients taking medication (e.g. steroids, antipsychotics) that may cause rapid glucose rise
6. Patients with acute pancreatic damage, including pancreatic surgery
7. Pregnancy
8. Presence of genetic, haematologic and illness-related factors that influence HbA1C and its measurement

Screening for Diabetes Mellitus

Because of the acute onset of symptoms, most cases of type 1 diabetes are detected soon after symptoms develop; screening is therefore not so important in this type of diabetes. According to some studies analysis of islet autoantibodies in some high risk individuals like those with previous transient hyperglycemia, or having family history of type 1 diabetes may be appropriate. But screening for type 1 diabetes in asymptomatic low risk individuals is not currently recommended.[20] On the other hand, in case of type 2 diabetes, situation is quite different. Individuals usually present with clinical signs and symptoms several years after the commencement of the disease process. Sometimes the complications may develop in individuals several years prior to the clinical diagnosis. Therefore screening is considered necessary in type 2 diabetes mellitus to identify asymptomatic individuals who are prone to develop diabetes or pre diabetes. Due to the increased chances of diabetes type 2 in children, screening is also considered important in them.[19,20]

Screening criteria in children for type 2 diabetes

Screening for type 2 diabetes is recommended, if a child is overweight with BMI >85th percentile for age and sex,

Table 1. Classification of diabetes mellitus[5]

1. Type 1 diabetes
2. Type 2 diabetes
3. Genetic defects of β-cell function
   a. Chromosome 20, HNF-4 a (MODY 1)
   b. Chromosome 7, glucokinase (MODY 2)
   c. Chromosome 12, HNF-1 a (MODY 3)
   d. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY 4)
   e. Chromosome 17, HNF-1 b (MODY 5)
   f. Chromosome 2 , NeuroD1 (MODY 6)
   g. Mitochondrial DNA and others
4. Genetic defects in insulin processing or insulin action
   a. Type A insulin resistance
   b. Leprechaunism
   c. Rabson-Mendenhall syndrome
   d. Lipoatrophic diabetes and others
5. Exocrine pancreatic defects
   a. Pancreatitis
   b. Trauma/pancreatectomy
   c. Neoplasia
   d. Cystic fibrosis
   e. Hemochromatosis and others
6. Endocrinopathies
   a. Acromegaly
   b. Cushing's syndrome
   c. Glucagonoma
   d. Pheochromocytoma
   e. Hyperthyroidism and others
7. Infections
   a. Congenital rubella
   b. Cytomegalo virus and others
8. Drugs
   a. Nicotinic acid
   b. Glucocorticoid
   c. Thyroid hormone
   d. Diazoxide
   e. β -adrenergic agonists
   f. Thiazides
   g. Dilantin
   h. γ- Interferon and others
9. Genetic syndromes associated with diabetes
   a. Down syndrome
   b. Klinefelter syndrome
   c. Turner syndrome
   d. Friedreich ataxia and others
10. Gestational diabetes mellitus

weight for height >85th percentile or weight >120% of ideal (50th percentile for height) and presents with any two of the following listed risk factors:

- Positive family history of type 2 diabetes in first or second degree relative(s)
- Certain races or ethnicities like Asian/Pacific islanders, American Indian, African-Americans, and Hispanics.

Table 2: Risk factors associated with type 2 diabetes mellitus

- Family history of diabetes (i.e. parents or siblings have diabetes)
- Overweight (BMI $\geq$25 kg/m^2)
- Habitual physical activity
- Race/ethnicity (e.g. African-American, Hispanic-American, Native Americans, Asian-Americans, and Pacific Islanders)
- Previously identified IFG or IGT or HbA1C 5.7-6.4%
- Hypertension ($\geq$140/90 mmHg in adults)
- HDL cholesterol $\leq$35 md/dl (0.90 mmol/l) and/or a triglyceride level $\geq$250 mg/dl (2.82 mmol/l)
- History of GDM or delivery of a baby weighing >9lbs
- Polycystic ovarian syndrome or acanthosis nigricans
- History of vascular disease

Table 3: Glucose levels enlightening normal condition, increase risk and diabetes mellitus

	Glucose level mg/dl (mmol/l)			HbA1c (%)
	Fasting	**Random**	**OGTT**	
Euglycemia (normal glucose levels)	<100 (5.6)	<200 (11.1)	<140 (7.8)	<5.7
Increased risk for diabetes	100-125 (5.6-6.9) (IFG)	-	140-199 (7.8-11) (IGT)	5.7-6.4 (Pre diabetic)
Diabetes mellitus	≥126 (7.0)	≥200 (11.1)	≥200 (11.1)	≥6.5%

- History of hypertension, dyslipidemia, polycystic ovary syndrome or acanthosis nigricans (conditions associated with signs of insulin resistance)
- Maternal history of diabetes or gestational diabetes mellitus

Table 4: Criteria for diagnosis of diabetes mellitus

1. HbA1C ≥6.5%, done in laboratory using a National Glycohemoglobin Standardization Program (NGSP) certified method and standardization to Diabetes Control and Complications Trial (DCCT) reference assay
2. Fasting plasma glucose (FPG) ≥126mg/dl (7.0 mmol/l). fasting is described as no calorie intake for at least 8 hours, only water is allowed
3. During an OGTT, 2 hours plasma glucose ≥200mg/dl (11.1mmol/l) as described by world health organization.
4. A random plasma glucose concentration of ≥ 200 mg/dl (11.1 mmol/l) with classical sign and symptoms of diabetes or with hyperglycemic crises

Screening should start at age of 10 years or at the start of puberty if puberty occurs at early age and it should be repeated after every two years. Fasting plasma glucose is most preferable method of screening.[21]

Screening criteria in adults for type 2 diabetes

1. Screening should be started in adults ≥ 45 years of age, especially if they have BMI ≥25 kg/m^2. If results are normal, the screening should be repeated at an interval of 3 years.
2. Adults should be screened early (< 45 years of age) and more frequently if they are overweight (BMI ≥25 kg/m^2) and have additional risk factors (Table 2)
3. For screening, either fasting plasma glucose (FPG) or 2 hour OGTT (75 gm glucose load) or HbA1C or all are considered suitable. [5,21] (Table 3)

Diagnosis of Diabetes Mellitus

Symptoms of diabetes mellitus include fatigue, nausea, frequent urination/polyuria, excessive thirst, obesity (mostly in type 2 diabetes), unusual weight loss, blurred vision, frequent infections, slow healing of wounds or sores. Sometimes no specific symptoms are present.[17] Although glucose analysis can be done in whole blood or serum but the most preferable sample is plasma. In case of normal hematocrit, glucose concentration is about 11% more in plasma as compared to whole blood. In whole blood, glucose decreases due to glycolysis. Such decreases in glucose concentration can miss the diagnosis of diabetes in a large proportion of population who have glucose concentrations near the cut points for diagnosis. In plasma and serum glucose variation is only about 1-2%.[22-24] However, immediate centrifugation in case of plasma prevents glycolysis while in case of serum, sample has to clot first and thus causes loss of glucose due to glycolysis.[5]
Diagnostic criteria of diabetes mellitus are mentioned in table 4. In the absence of unequivocal hyperglycemia, criteria 1-3 should be confirmed by repeat testing. For example, if only one test like HbA1C or FPG or OGTT is performed, then in order to confirm the diagnosis, the same test should be repeated. Sometimes, if the results of FPG are <7 mmol/l, but still there is strong suspicion of diabetes, then an OGTT should be performed. If the results of two different tests of same patient are provided, and both are above the diagnostic values of diabetes mellitus, then the diagnosis of diabetes is confirmed and there is no need to further repeat the test. In cases where the results of two tests in same patient are opposite to each other (one above the diagnostic value of diabetes while second one in normal range), then the test whose result is above the diagnostic cut point should be repeated. The diagnosis in such a situation should be confirmed on the basis of repeat test results. Considering the pre-analytical and analytical variabilities, sometimes in contrast to the first test result, the repeat test may show a value within the reference range. This is most common in case of OGTT than FPG and least common in case of HbA1C. In such a situation, patient should be followed closely, and it is recommended that the test should be repeated within 3-6 months.[5]

Prognosis/Control of Diabetes

HbA1C is a widely used method to calculate the last 2-3 months diabetic control. In diabetics, it should be maintained at or below 7%. As the value of HbA1C increases, there will be more risk of developing microvascular and to lesser extent macrovascular complications.[5,20] It is generally recommended that HbA1C should be repeated at an interval of six months in patients who respond well to their treatment and have good glucose control. If diabetics have a bad glycemic control or if the anti-diabetic medication is to be changed, the test should be repeated at an interval of three months.[20]

Oral glucose tolerance test (OGTT)

Indications: Equivocal blood glucose levels, pregnancy or epidemiological studies.
Preparation: Patient should be advised to take unrestricted diet (greater than 150 g of carbohydrate daily) and maintain usual physical activity at least for three days before the test. Reasonable (30-50g) carbohydrate containing meal should be consumed on the evening before the test. The test should be preceded by an overnight fast of 8-14 hours, during which water may be allowed. Smoking is not permitted during the test. The presence of factors that influence interpretation of the results of the test must be recorded (e.g. medications, inactivity, infection, etc.).
Procedure: After collection of the fasting blood sample, the subject should drink 75 g of anhydrous glucose or 82.5 g of glucose monohydrate (or partial hydrolysates of starch of the equivalent carbohydrate content) in 250-300 ml of water over a course of 5 minutes. For children, the test load should be 1.75 g of glucose per kg body weight up to a total of 75 g of glucose. Timing of the test is from the start of the drink. Blood samples must be collected 2 hours after the test load. Unless the glucose concentration can be determined immediately, the blood sample should be collected in a tube containing sodium fluoride (6 mg/ml whole blood) and immediately centrifuged to separate the plasma; the plasma should be frozen until the glucose concentration can be estimated.[3] Results are interpreted according to table 3.

References

1. Shoback, edited by David G. Gardner, Dolores shoback (2011).Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. pp. Chapter 17
2. Saltiel AR, Kahn CR. Insulin signaling and the regulation of glucose and lipid metabolism. Nature. 2001; 414: 799-806
3. Report of a WHO consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. World Health Organization Department of Noncommunicable Disease Surveillance Geneva. 1999

4. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010; 87: 4-14
5. American diabetes association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2012; 35(S1): S64-S71).
6. Mølbak AG, Christau B, Marner B, Borch–Johnsen K, Nerup J. Incidence of insulin–dependent diabetes mellitus in age groups over 30 years in Denmark. Diabet Med. 1994;11:650–55
7. Zimmet PZ, Tuomi T, Mackay R, Rowley MJ, Knowles W, Cohen M et al. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. Diabetic Med 1994; 11: 299–303
8. Humphrey ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet P. Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult–onset diabetes mellitus. Diabetic Med 1998;15: 113– 19
9. McLarty DG, Athaide I, Bottazzo GF, Swai ABM, Alberti KGMM. Islet cell antibodies are not specifically associated with insulin–dependent diabetes in rural Tanzanian Africans. Diabetes Res Clin Pract. 1990;9: 219–24
10. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA et al. Predicting type I diabetes in first–degree relatives using a combination of insulin, GAD, and ICA512bsdc/IA–2 autoantibodies. Diabetes 1996;45: 926–33
11. Japan and Pittsburgh Childhood Diabetes Research Groups. Coma at onset of young insulin–dependent diabetes in Japan: the result of a nationwide survey. Diabetes 1985;34: 1241–46
12. Hother–Nielsen O, Faber O, Sørensen NS, Beck– Nielsen H. Classification of newly diagnosed diabetic patients as insulin–requiring or non–insulin–requiring based on clinical and biochemical variables. Diabetes Care. 1988; 11: 531–37
13. Betterle C, Zanette F, Pedini B, Presotto F, Rapp LB, Monsciotti CM et al. Clinical and subclinical organ– specific autoimmune manifestations in type 1 (insulin– dependent) diabetic patients and their first–degree relatives. Diabetologia. 1983; 26: 431–36
14. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E et al. Insulin resistance and insulin secretory dysfunction as precursors of non–insulin– dependent diabetes. Prospective Study of Pima Indians. N Engl J Med. 1993; 329: 1988–92
15. Withers, D. J. et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature. 1998; 391: 900–904
16. Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ et al. Prevalence and determinants of glucose intolerance in a Dutch population. The Hoorn Study. Diabetes Care. 1995; **18:** 1270–73
17. Kumar BV, Abbas AK, Fausto N, Mitchell R. Diabetes mellitus. In: Kumar BV, Abbas AK, Fausto N, Mitchell R, editors. Robbins basic pathology, 8th edition. Philadelphia: Saunders; 2007:775–787
18. WHO recommendations. Use of Haemoglobin A1c (HbA1c) in the diagnosis of diabetes mellitus in the UK. 2011
19. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, Lernmark A, Metzger BE, Nathan DM. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes care. 2011;34: e61-e99
20. American diabetes association. Standards of medical care in diabetes-2011. Diabetes care. 2011; 34(1): S11-S61
21. American diabetes association. Standards of medical care in diabetes-2006. Diabetes care. 2006;29(1):S4-S42
22. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In: Sonnenwirth A,Jarett L,eds.Clinical laboratory methods and diagnosis . St. Louis : C.V. Mosby Co .; 1980 :149–92
23. Gambino R, Piscitelli J, Ackattupathil TA , T heriault JL , Andrin RD, Sanfilippo ML , Etienne M . Acidification of blood is superior to sodium fluoride alone as an inhibior of glycolysis. Clin Chem 2009; 55:1019–21
24. Carstensen B, Lindstrom J, Sundvall J, Borch-Johnsen K, Tuomilehto J. Measurement of blood glucose: comparison between different types of specimens. Ann Clin Bio chem. 2008 ;45:140–8