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DIABETES MELLITUS: LABORATORY DIAGNOSIS

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ABSTRACT

Diabetes mellitus is a group of disorders and disturbances leading to a variety of alterations. Several studies have been carried out on the role of laboratory diagnosis for diabetes mellitus. The most common types of diabetes (type-1 and type-2) are polygenic whereas neonatal diabetes mellitus (NDM) and maturity-onset diabetes of the young (MODY) are monogenic. Purpose of diabetes testing is to screen for hyperglycemia, diagnose diabetes/ prediabetes, control the glucose levels over time and find and monitor the dysfunctions/ complications. Management to control or delay/ prevent type-2 diabetes by losing a modest amount of body weight in overweight or obese people is quite effective approach. However, proper diagnosis of diabetes and prediabetes requires FPG (fasting plasma glucose) test and A1C (hemoglobin A1C or Hb Alc) test, though RPG (random plasma glucose) test can be done for some cases. Fasting, HbA1c and OGTT (oral glucose tolerance test) glucose levels have been determined with excellent laboratory expertise in various recent studies. However, the existing diagnostic tests can be compared with the new diagnostic tests including glycated albumin (GA) in conditions wherein better and more accurate glycemic status is required. Hopefully, the future laboratory diagnostic techniques will help tremendously for therapeutic efficacy and survival. Hence, there is an urgent need to search for additional tests or to replace the HbA1c and OGTT for up-to-date laboratory diagnosis of diabetes mellitus.

KEYWORDS: Diabetes mellitus; laboratory diagnosis; fasting, random, HbA1c, OGTT and GA tests

Abbreviations: A1C: hemoglobin A1C; BMI: body mass index; BUN: blood urea nitrogen; CMP: comprehensive metabolic panel; DCCT: diabetes control and complications trial; eAG: estimated average glucose; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; GA: glycated albumin; GDM: gestational diabetes mellitus; HbA1c: Hemoglobin A1C; HDL: high density lipoprotein; LDL: low density lipoprotein; MODY: maturity-onset diabetes of the young; NDM: neonatal

diabetes mellitus; OGTT: Oral glucose tolerance test; PG: plasma glucose; PNDM: permanent neonatal diabetes mellitus; RBCs: red blood cells; RPG: random plasma glucose; T1-DM: type 1 diabetes mellitus; T2-DM: type 2 diabetes mellitus; TNDM: transient neonatal diabetes mellitus

INTRODUCTION

Diabetes mellitus is a group of disorders leading to a variety of pathophysiological alterations. There several studies have been carried out in connection with the role of laboratory diagnosis of diabetes mellitus considering several perspectives (Pollak *et al*., 1981; Hussain, 1994; Kawakami *et al*., 1997; Roden *et al*., 2000; Hussain *et al*., 2007a, 2007b; Lee *et al*., 2008; Maruyama *et al*., 2008; Sohail and Hussain, 2008, 2009, 2013, 2017; Kalra, 2009; Nayak *et al*., 2012; Ostrowska *et al*., 2013; Sohail *et al*., 2013, 2019; Benbaibeche *et al*., 2014; Kim *et al*., 2015; Ahmadi *et al*., 2017; Romera *et al*., 2020). Diabetes mellitus appears in three main forms: type 1 diabetes mellitus (T1-DM), type 2 diabetes mellitus (T2-DM) and type 3 or gestational diabetes mellitus, and the most common type of diabetes, however, is T2-DM wherein obesity, physical inactivity, family history, age and certain ethnicities are prevalent risk factors manifesting a variety of systemic pathophysiological alterations (Sohail, 2015).

The most common types of diabetes (type-1 and type-2) are polygenic whereas neonatal diabetes mellitus (NDM) (Kanakatti Shankar *et al*., 2013; Letourneau *et al*., 2017) and maturity-onset diabetes of the young (MODY) are monogenic (Kanakatti Shankar *et al*., 2013; Pihoker *et al*., 2013; Rubio-Cabezas *et al*., 2014). About half of those having NDM have the condition for lifelong time and called as permanent neonatal diabetes mellitus (PNDM), whereas in the remaining those with NDM the condition occurs temporarily in infancy and may reappear sometime later in life called as transient neonatal diabetes mellitus (TNDM). Monogenic diabetes is diagnosed by genetic testing using blood or saliva DNA, and the most forms of NDM and MODY are caused by autosomal dominant mutations.

Genetic testing is recommended where mutations in KCNJ11 and ABCC8 genes encoding ATP-dependant potassium channel of the β cell occur most commonly in neonatal diabetes (Gaál and Balogh, 2019). Case study of NEUROD1-MODY- a rare form of monogenic diabetes caused by mutations in Neuronal differentiation have been reported worldwide and one of those cases was revealed in a Latin American family (Abreu *et al*., 2019) but there is still a little know how about the actual involvement of mutations in NEUROD1 in monogenic diabetes and its clinical impact. However, the MODY is though lower in frequency in diabetic patients and a clinically heterogeneous group of monogenic disorders having ß-cell dysfunction, it has recently been attempted for laboratory diagnosis by Sanger DNA sequencing for identifying MODY-related gene mutations (Jang, 2020).

Purpose of diabetes testing is to screen for hyperglycemia, diagnose diabetes/ prediabetes, control the glucose levels over time and find and monitor the dysfunctions/ complications. Management to control or delay/ prevent type-2 diabetes by losing a modest amount of body weight in overweight or obese people is quite effective approach. However, proper diagnosis of diabetes and prediabetes requires i) FPG (fasting plasma glucose; preferably in morning after fasting of more than 10 hours) test done after no eating or drinking except a little water sips, and ii) A1C (hemoglobin A1C or glycohemoglobin) test, though RPG (random plasma glucose; no overnight fasting) test

can be done for some cases where diabetic symptoms are present and the health care personnel do not like to wait long until get results for A1C. Fasting, HbA1c, OGTT glucose levels were determined with excellent laboratory expertise in various recent studies (Jones *et al*., 2020; Rojo-Martínez *et al*., 2020; Romera *et al*., 2020).

The existing diagnostic tests might be compared with the new diagnostic tests including glycated albumin (GA) in conditions where better and more accurate glycemic status is required. Hopefully, the future laboratory diagnostic techniques will help tremendously for therapeutic efficacy and survival.

TESTING DIABETES MELLITUS

Diabetes type-1 that is usually diagnosed in children and adults manifests symptoms and testing for family members, and test is carried out in those with symptoms or without symptoms since it is a familial type. Diabetes type-2 is tested by checking the age (45 and above 45; between 19 and 44 overweight or obese showing one or more risk factors) and if they are women having gestational diabetes (American Diabetes Association, 2016). Children are tested who are overweight or obese and have any risk factors for diabetes type-2 (American Diabetes Association, 2016) including their low birth weight and if their mothers were diabetic while pregnant with them.

Third type of diabetes- gestational diabetes can be tested with glucose challenge test (or glucose screening test) done without fasting during 24 to 28 weeks of their pregnancy (American Diabetes Association, 2016, 2018) and if the glucose level is higher than 140 then oral glucose tolerance test (OGTT) is carried out every hour for 2-3 hours (that does require fasting for 8 hours or more) or both of these tests. High glucose level at any two or more test times (after one hour, two hours or three hours) indicates gestational diabetes.

TESTS FOR SCREENING AND DIAGNOSIS

Diabetes testing in general is carried out a) to monitor glucose control and diabetes on regular basis to find diabetes/ diabetic complications sooner as a management and preventive approach, b) when a person shows signs and symptoms of diabetes mellitus, c) when a person has risk factors associated with diabetes, d) when a person reveals with acute condition as an emergency. However, screening gestational diabetes has different criteria.

Table 1. Fasting plasma glucose (FPG) test.

Fasting glucose	**Condition**
70-99 mg/dL (3.9-5.5 mmol/L) Max: 110 mg/dL (6.1 mmol/L)	Normoglycemia
110-126 mg/dL (6.1-7.0 mmol/L)	Prediabetes
≥ 126mg/dL (≥7 mmol/L) & above; tested more than one time	Diabetes mellitus

Urine analysis is also done mainly to test glucose, protein, ketones etc while routine checking up of patients. This may help to have idea about the problems a patient might have and identifying the underlying cause. Different tests are performed for screening and diagnostic purpose. Confirmation with the single test used to diagnose diabetes is not enough. A second measurement is essentially required unless there are quite evident symptoms of diabetes (American Diabetes Association, 2018). These test are mainly

fasting plasma glucose (FPG; measured after 8-12 hour fasting; (Table 1), random plasma glucose (RPG), A1C that measures an average blood glucose level over past 2-3 months (Table 2), and OGTT (2-hour glucose tolerance test; fasting blood test, followed by 75-gram glucose drink and testing sample two hours thereafter, (Table 3).

If symptoms of diabetes are present, a random plasma glucose test is performed by drawing blood sample and measuring glucose without keeping in fasting. This is considered as part of a CMP (comprehensive metabolic panel). Result of higher than 200 mg/dL (11.1 mmol/L) indicates diabetes and can be confirmed by further tests. The result of 200 mg/dL (11.1 mmol/L) around two hours after eating is considered as prediabetes. However, the value of 200 mg/dL (11.1 mmol/L) one hour after eating is considered as normal.

The A1C test (based on attachment of glucose with hemoglobin) sometimes called as hemoglobin A1C (HbA1c), glycohemoglobin or glycated hemoglobin test serves as a primary test for the management of diabetes, and used to diagnose type-2 diabetes and prediabetes (Gillett, 2009). Hence, higher glucose blood level means more glucose attached with hemoglobin over the past 3 months. It is shown in percentage (normal level of A1C is around 5.7 percent). A1C range of 5.7 to 6.4 percent is the diagnosis for prediabetes, and higher A1C reveals greater risk of diabetes (Table 2).

The A1C may vary (a little higher or lower) from the same blood sample (Penttilä *et al*., 2016). Hence, more information can be obtained about the precision of A1C test and more research studies are needed to be conducted. The A1C is recommended at least twice a year for individuals with diabetes, though A1C might be checked for those not following the treatment goal (American Diabetes Association, 2018).

The vein blood taken and sent for the glycohemoglobin standardization method provides results as according to DCCT (diabetes control and complications trial). The health authorities may decide which other tests beside A1C be done for diagnosing diabetes and prediabetes. The A1c test does not require fasting for 8 hours and should not be implemented for diabetic diagnosis in pregnant women, and people with iron or vitamin B12 deficiency anemia, chronic liver or kidney disorders and in other conditions where A1C gives false results. The A1C test can be employed to find risk factors in undiagnosed diabetes before the start of pregnancy. Testing early in pregnancy in 24-28 weeks by glucose challenge test or glucose screening test is preferred to be done by OGTT (oral glucose tolerance test, Table 3) in case glucose levels are found 140 or higher.

Purpose of having A1C test is to find type-2 diabetes, to find prediabetes, provide opportunity to do lifestyle changes (such as weight loss and keeping physically active during most of the days of week) for delaying or preventing diabetes type-2, and decide the treatment in case diabetes is diagnosed.

Table 2. HbA1c test.

Subjects for diagnosis	**HbA1c test**	
	DCCT (%)	mmol/mol
Normoglycemia	<6.0	<42
Prediabetes	6.0-6.4	42-46
Diabetes mellitus	> 6.5	≥48

The A1C test is not used for diagnosing type-1 diabetes. This test is performed following various procedures and hence inter-laboratory results may vary, and so can be

used as bedside care or doctor's office to monitor the treatment with drug therapies or lifestyle. But standard methods should be employed for diagnostic and screening purposes.

If there are no apparent symptoms of diabetes but the A1C test indicates diabetes or prediabetes, the test should be repeated on a different day using the A1C test or one of the other diabetes tests for the confirmation of diagnosis (American Diabetes Association, 2018).

It is possible that a person's A1C shows the diagnosis of diabetes and blood glucose test does not show the diagnosis of diabetes. Reverse can also happen. In such cases tests are repeated. However, A1C and blood glucose tests showing different diagnosis might be in the early stage of diabetes considered as swings of prediabetes or progressing complications of diabetes that requires close check-up. Eating, exercise, stress, change in diet, sickness, changes in lifestyle etc influence the blood levels of glucose. A1C can be reduced to some extent by reducing the complications/ risk factors of diabetes and prediabetes. However, changes in A1C tests are less compared to those in FPG or OGTT tests.

Other changes that occur in RBCs and hemoglobin e.g. life span of RBCs, hemodialysis, sickle cell anemia, transfusion, erythropoietin treatment etc may change the A1C levels. It becomes quite difficult to diagnose diabetes and prediabetes in those people who are African, Mediterranean, or Southeast Asian descent especially when without symptoms since they have different types of hemoglobin variants and hence their A1C and blood glucose test levels may vary from each other. However, A1C test levels of glucose measured considering their hemoglobin variants in some of the people may not vary from blood glucose test results.

Blood test results especially using fasting test (Table 1) and OGTT (Table 3) may vary more compared to those done by A1C test due to sample handling, temperature and equipment condition/ efficiency. However, tests may be repeated and quite high levels may be interpreted for diagnostic purpose.

Controlling blood glucose strictly for initial years might be beneficial. However, with the emergence of more complication, less strict glucose control might be more beneficial e.g. strict control of A1C sometimes may lead to complications of hypoglycemia. Furthermore, less strict control of A1C might be necessary for people having chronic kidney disease, diabetic neuropathy, cardiovascular complications and hypoglycemia unawareness (inability to sense hypoglycemia/ severe hypoglycemia) and those with limited life expectancy.

Estimated average glucose (eAG) can be calculated from A1C levels. It is expressed in mg/dL but does not match with daily glucose estimations since it is a long term average estimation though the more accurate eAG values are obtained if A1C covers only 30 days instead of longer period of several months. Hence A1C or eAG do not provide values for sudden or instant increase or decrease in blood glucose.

Clinical trials for A1C test and diabetes are conducted by various institutes to learn how can we improve A1C test results considering the life span of RBCs that might be longer or shorter, to search for other tests that might be better than A1C for certain individuals, and to investigate how the relationship between A1C and blood glucose change for various racial/ ethnic groups.

While studying the diagnostic criteria, A1c and fasting glucose tests showing 6.9% of subjects as prediabetes were categorized as having diabetes based on 2-h plasma glucose $\geqslant$200 mg/dL (Menke *et al*., 2018). Laboratory tests for these subjects

with diabetes based on 2-hour plasma glucose ⩾200 mg/dL indicated hypertension, low HDL cholesterol, high triglycerides, elevated alanine aminotransferase and albuminuria present more likely whereas no other cardiometabolic risk factors found associated (Menke *et al*., 2018).

Table 3. Oral glucose tolerance test (OGTT).

Glucose level 2 hrs after 75 gram glucose drink	**Condition**
140 mg/dL (7.8 mmol/L)	Normal glucose tolerance
140-199 mg/dL (7.8-11.1 mmol/L)	Prediabetes
200 mg/dL (11.1 mmol/L) & above; tested more than one time	Diabetes mellitus

Screening for type 2 diabetes is not recommended but screening for abnormal blood glucose or type-2 diabetes before signs and symptoms appear leads to early diagnosis and treatment. The diagnosis can be confirmed with a fasting plasma glucose level (126 mg per dL or higher), A1C level (6.5% or higher), random plasma glucose level (200 mg per dL or greater); or otherwise a 75-g two-hour oral glucose tolerance test with glucose level as 200 mg per dL or higher. It was found that the lifestyle and pharmacologic approaches helped decreasing the progression to diabetes in patients with impaired fasting glucose or impaired glucose tolerance (Pippitt *et al*., 2016). A fasting plasma glucose criterion and an HbA1c criterion were used for diagnosing the patients with diabetes mellitus (Hong *et al*., 2016).

Performance of plasma glucose (PG) as well as HbA1c for detecting diabetes was evaluated and it was suggested that though HbA1 level above 6.5% has been recommended for diagnostic purpose by American Diabetes Association, it is important that over-diagnosis be avoided while having only elevated HbA1 (Xu *et al*., 2016). It was noted that levels of BMI, cholesterol, waist circumference and other cardiovascular risk factors in diabetes were higher by testing HbA1 alone compared to those tested by oral glucose tolerance test (OGTT) (Xu *et al*., 2016). Furthermore, FPG together with HbA1c identified diabetics exceeding 85% compared to those identified by any of the three tests (HbA1c, FPG, 2-h PG) (Xu *et al*., 2016).

Measuring fasting plasma glucose (FPG) and HbA(1c) in combination are useful to preoperatively identify coronary patients having unknown diabetes (Tekumit *et al*., 2010) since FPG alone was not found sufficient for diagnosing almost half of the patients having dysglycemia. Hemoglobin A1c with fasting plasma glucose and 2-h postchallenge glucose was compared for women with recent GDM (gestational diabetes mellitus) (Kim *et al*., 2011) that showed fair agreement between A1C and glucose levels for detecting abnormal glucose tolerance test.

CONCLUSIONS

The main test for monitoring diabetes type-1 for setting a schedule or modifying the schedule of medication relates to the patients for monitoring their own blood several times a day as according to the instructions of the consultant and to determine up and down fluctuations of glucose levels. Some of the women with gestational diabetes and diabetes type-2 may also require similar procedure for monitoring their blood glucose levels.

Test for monitoring diabetics and prediabetics is A1c serving a test for measuring the average blood glucose levels over the past 2-3 months and calculating eAG several time a year. Glucose control can also be measured by other tests including fructosamine test (to evaluate glucose levels during the last 2-3 weeks), 1,5 Anhydroglucitol (for detecting high levels of glucose in last 1-2 weeks), monitoring kidney function (creatinine clearance, urinary albumin (microalbumin), creatinine, cystatin C, eGFR (estimated glomerular filtration rate), CMP, BUN (blood urea nitrogen) etc), and monitoring cholesterol and other components of lipid profile (HDL cholesterol, LDL cholesterol, triglycerides etc), and glycated albumin (GA) (that is a useful alternative to HbA1c under the conditions when latter does not reflect glycaemic status quite accurately).

Better diagnostic tests are essentially required to improve early confirmation of diagnosis, which may provide better opportunity for therapeutic efficacy and survival. Hence, there is urgent need to check the existing diagnostic tests including HbA1c for diagnostic efficacy and compare these with the glycated albumin (GA) and such other tests that may serve as additional tests or replace the HbA1c and OGTT.

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