

Diabetes Mellitus: the Role of the Laboratory- an Update

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Historical background

Diabetes mellitus has been known to mankind since ancient times .The Egyptian Papyrus described a disorder in which “honeyed urine” was passed - The first clear account of diabetes mellitus was written by the Roman, Aretaeus¹ A.D.81-138 who described it as a “mysterious affliction” leading to a “melting down of flesh and limbs into the urine Life is short, disgusting and painful thirst unquenchable and death inevitable.”

The discovery of insulin in 1921 was one the great achievements of 20th century medicine, it became available for clinical use 2 years later².

Since the advent of insulin and intravenous fluid replacement therapy, the morbidity and mortality from coma in patients with diabetes mellitus (DM) has been significantly reduced . However there is still a very high incidence of chronic complications in DM. This disorder is one of the commonest cause of blindness . Diabetic nephropathy is a major clinical problem and renal failure is reported to be the cause of death in over 20% of type 1 diabetics³.

A well equipped and well run laboratory is a great benefit for the physician as regards Diabetes Mellitus in the following areas:

1. Diagnosis of DM.
2. Glycaemic control.
3. Detecting long-term complications of DM.
4. Diabetic comas.

1. Diagnosis of Diabetes Mellitus Is Diabetes present?

If patient presents with:

- a. Clinical symptoms suggestive of D.M e.g. (polyuria, polydipsia ,weight loss)
- b. Glycosuria on routine urine testing.
- c. A strong family history.
- d. A suggestive obstetric history.
- e. Other disorders associated with diabetes mellitus , e.g. retinopathy, peripheral vascular disease or peripheral neuropathy. Then it is obligatory to measure fasting plasma glucose.

Further action should be taken after assessing the patient in the context of the table given below^{4,5}.

Table 1. Diagnosis of diabetes mellitus.

Diagnosis of diabetes mellitus and other categories of hyperglycaemia (according to the WHO recommendations 1997) ^{5,6}				
	Fasting		2 hr. post glucose load	
	Venous sample glucose mg/dl		Venous sample glucose mg/dl	
	Whole blood	Plasma	Whole blood	Plasma
Impaired				
fasting glycaemia (IFG)	100-110	110-140	<120	<140
Impaired glucose tolerance (IGT)	<110	<126	120-180	140-200
Diabetic	>110	>126	>180	>200

Ideally a patient should be assessed by both, a fasting sample and 2-hour value. If this is not done then it must be accepted that a certain number of people will be missed^{6,7}.

An additional new category Impaired Fasting Glycaemia(IFG) has been added, although its range is quite narrow it does highlight that some subjects have non-diabetic fasting values which are above the absolutely normal so they may need to be monitored, but the full significance of this group needs further evaluation.

If subsequently the patient falls into the Impaired The patient should have been on a normal diet for at least the last three days.

This especially means a normal carbohydrate intake. During the test the patient should be resting and should not be smoked.

- Any drugs the patient is using have to be noted as certain drugs like steroids, diuretics, oral contraceptives may be important.

There is no justification for taking half-hourly samples for two hours. Diagnosis may be made on the basis of fasting and 2 hour blood sample, with one hour sample taken to provide additional information. There is also no justification for proceeding with the glucose tolerance test when the fasting blood glucose is raised. Measurement of glycosylated protein is not included in the diagnostic criteria^{7,8}.

The procedure for OGTT

a. The patient fasts overnight (at least 10 hours). Water is allowed.

- b. A venous sample is taken for plasma glucose and a urine sample is collected by the double void technique.
 - c. A solution containing an equivalent of 75g of glucose in 300ml. (N.B. This solution should be drunk slowly, the solution should not be too concentrated as excessive hyperosmolality may lead to vomiting and even without vomiting to poor absorption).
 - d. The patient should sit quietly and not smoke.
 - e. Further blood and urine samples are taken at 2hours after the ingestion of glucose.
- The interpretation of the oral glucose tolerance test is shown in Table 2.

Table 2.

Interpretation of oral glucose tolerance test		
Venous plasma glucose mg/dl		
	Fasting level	Two hour level
Diabetes unlikely	<110	<140
Impaired glucose tolerance	<110	140-200
Diabetic	>110	>200

N.B. It should be noted that patients with impaired glucose tolerance should be followed up annually as upto 4% per annum develop overt D.M.

2. Laboratory aspects of Diabetic Control Blood Glucose

A poor glycaemic control is the major contributor to the development of complications^{4,5}. There are various methods of monitoring but glucose measurement in blood and for urine is most widely used. Blood glucose

measurement although being indispensable for the management of DM , it can have its drawbacks and limitations and these should be clearly understood.

Many methods are available for the measurement of blood glucose. When noting the result certain considerations must be looked into i.e., source (capillary or venous), plasma (14% higher) or whole blood, collection made in a suitable preservative e.g. sodium fluoride (inhibits glycolysis).

In Type I D.M.

These patients can present monitoring problems; their glucose can be highly variable. In addition fasting and random samples sometimes give no indication of the glycaemic fluctuations at home or at work.

In these patients a blood glucose series over a 24hr period during a normal day gives the most information. This may be done while the patient is at home using finger prick capillary blood glucose with reagent strips and a reflectance meter provided the meter has been calibrated and the patient is taught the correct procedure. This can only be done when a liaison is established between a laboratory and the patient.

Alternatively, it can be performed when the patient is in hospital but reasonably active. In these patients the blood glucose measurement is an indispensable tool in spot checks for hypoglycaemia and

hyperglycaemia.

In Type II D.M.

In these subjects the fasting blood glucose fairly accurately represents the mean blood glucose concentration for the previous 24 hrs. Post-prandial glucose values in these patients also gives a good indication of the glycaemic control and whether 'or not their treatment is appropriate.

Urine Glucose Testing

Urinary testing for glucose is still unfortunately, a popular test both in type I and type II DM. The major methods for the detection of urinary glucose in routine

1. Clinitest tablets- containing Benedict's reagent (detect not only glucose but all reducing agents - false positive. Admittedly these are no longer available.

Interpretation of HbA1c in Diabetic Patients.

N.B. The ranges of HbA1c may vary to a minor extent in different kits.

HbA1c value	Interpretation
Less than 6%	Over-treatment
6.0 to 8.0%	Very good control
8.0 to 10 %	Poor control
10 to 12 %	Modify therapy
Above 12%	Patient will require careful monitoring and modification of diet therapy.

2. Glucose oxidase: peroxidase dye impregnated strips which change colour in presence of glucose e.g. Clinistix or Diastix. These can sometimes give a false positive, e.g. with ascorbic acid.

However urine testing may not be a particularly reliable means of assessing or obtaining glycaemic control for the following reasons;

- It is retrospective, being a reflection of what blood glucose was two hours previously. So it is not at all desirable to adjust the insulin dosage using urine testing.
- Urine testing is dependent upon the renal threshold. If the glomerular filtration rate (G.F.R.) is raised as it is in pregnancy glycosuria may occur with blood glucose of less than 180mg/dl. If the G.F.R. is lowered, as is the case in elderly patients and in renal disease there is reduced rate of delivery of fluid to tubules and a higher concentration of glucose may be reabsorbed. Consequently there may be no glycosuria even if plasma glucose levels exceed 230mg/dl.
- Urine testing gives no information about the presence or not of hypoglycaemia.

Glycosylated Haemoglobin (HbA1c)

Despite increasing understanding of the pathogenesis and therapy of diabetes mellitus, the most important question concerning the relationship between the degree of metabolic control and the occurrence of long -term complications of diabetes was hampered by lack of satisfactory methods.

Since the advent of glycosylated haemoglobin the monitoring of metabolic control has been considerably aided 8-10.

Human haemoglobin is heterogeneous. In normal adults and children older than six months about 90% of the haemoglobin is HbA, which consists of a pair of, α and β polypeptide chains attached to the haem molecule. HbA₂ and HbF comprise approximately 2.5% and 0.5% of the total haemoglobin respectively. In addition to these there are a further three minor components HbA_{1a}, HbA_{1b} and HbA_{1c}. These minor haemoglobins exhibit a faster chromatographic separation than the main band of haemoglobin A and are collectively referred to as "fast haemoglobin" or HbA₁ or "glycosylated haemoglobin". These minor haemoglobins differ from HbA by having a carbohydrate moiety attached to the N-terminal of the β chain and account for approximately 5.5-8.5% of the total haemoglobin. HbA_{1c} is a term reserved for the HbA, which has a glucose molecule, attached to the N-terminus by a ketamine linkage (glycosylation). The other minor haemoglobins either have an attachment of a sugar phosphate to the N-terminus of the beta-chain or they may be a further modification of HbA_{1c}. HbA_{1c} can be elevated as much as four-folds in diabetic erythrocytes (whereas both HbA_{1a} and HbA_{1b} are not as reliably elevated) in fact the HbA_{1c} is most affected by the mean blood glucose concentration.

Studies indicate that HbA_{1c} is formed slowly and continuously throughout the life span of the erythrocyte furthermore it is formed non-enzymatically and essentially irreversibly. The rate of glycosylation and amount of HbA_{1c} formed in the erythrocyte is proportional to:

1. The mean plasma glucose
2. The life span of the erythrocyte

Thus the levels of HbA_{1c} and HbA will provide an integrated measure of blood glucose levels over the preceding two to three month's period, (in other words the average exposure to glucose during it's lifespan) and this is the rationale for using HbA_{1c} as for the assessment of diabetic control,^{2,3}.

Diphosphoglycerate (2,3,DPG) binds to the N-terminal valine of the beta chain of haemoglobin and this being the same site where glucose attaches to form HbA_{1c}, there is a competition between glucose and 2,3 DPG. Consequently in the presence of increased concentrations of HbA_{1c}, there is less 2,3 DPG bound to the molecule and there will be diminished delivery of oxygen to the tissues. This may contribute to the hypoxic complications of DM and lead to the microangiopathic changes.

It should be noted that HbA_{1c} and the other minor components i.e. HbA_{1a} and HbA_{1b}, are more electronegative than the main band of HbA and this property enables us to separate them from the major HbA components.

There are several methods now available for the determination of HbA_{1c};

1. Electrophoresis.
2. Cation Exchange Chromatography.
3. Colorimetry. (Mostly used in routine laboratories, can be automated)
4. Isoelectric focusing.

Advantages of using Glycosylated Haemoglobin as an index of diabetic control

1. A single determination can substitute for several glucose determinations made at different time intervals.
2. It does not vary immediately after meals or exercise thus samples can be taken at any time during the day.
3. Serial determinations may be used to evaluate the relationship of blood glucose control in diabetes with the development of complications.
4. Useful in confirming home glucose monitoring particularly in children, where compliance may be poor.
5. Has proved to be a very useful tool in pregnant diabetics. In fact, since its advent there has been downward trend in obstetric mortality because of better monitoring and stricter diabetic control at the time of conception as well as during pregnancy. A HbA_{1c} value greater than 12% at 12 weeks of

pregnancy should alert the obstetrician to the possibility of major congenital abnormality in the foetus.

Limitations in the use of HbA1

1. As HbA1 levels in the blood depends on red cell turnover, values are reduced in the following conditions^{11,12}.
 - a. Haemolytic anaemia.
 - b. Splenomegaly.
 - c. Patients with haemochromatosis undergoing repeated venesection.
2. Erroneous results may result from high levels of HbF in conditions such as f3-Thalassaemia.
3. High values are obtained in iron deficiency anaemia, may be due to increased glycosylation.
4. Falsely high or low results can occur in patients who had recent blood transfusions.
5. Its role as an aid in the diagnosis of D.M. is not as clear as its role in the management .It may be of some use in confirming the diagnosis of diabetes mellitus in longstanding hyperglycaemia , but quite often it is normal in newly developed diabetes and it may be normal in patients with minor disturbances of glucose tolerance.
6. Should not be used to diagnose hypoglycaemia although recurrent low values may indicate risk of hypoglycaemia. Glycosylated Proteins

Methods are available for the measurement of serum Fructosamine^{13,14}. which is an indicator of glycosylated protein, it is expressed as mmol/l. Since serum proteins generally have a half-life of about 20 days, this is an index of short term diabetic control over the preceding 2-3 weeks. As such it may be suitable to use as a monitoring tool in diabetic pregnancies. Its advantages are that it can be automated therefore having good analytical precision and it is not expensive. However it may be unreliable in patients with altered protein metabolism i.e. liver disease or severe illness although it has been suggested that the result should be corrected for serum albumin.

Other Glycosylated Methods

Glycosylation of other proteins also is determined by its contact with a given level of glucose e.g. low density lipoprotein, high density lipoprotein, lens and glomerular basement membrane¹⁵.

Glycosylation of collagen from diabetic foot scrapings has been estimated and found to correlate very well with the HbA 1 level in diabetic patients with peripheral vascula: disease and peripheral neuropathy.

But these are not feasible as routine monitoring tests especially when HbA I is available.

7. Long term Complications of DM

a) Nephropathy

A significant number of diabetics (especially those diagnosed before the age of thirty) die from diabetic nephropathy .It is now well established that early changes in the diabetic kidney may be significantly reduced and even reversed by excellent diabetic control¹⁶ Proteinuria is the clinical hallmark of diabetic nephropathy.

Clinical proteinuria is defined as a concentration of greater than 0.5 g/ 24 hours. Albustix may detect this, in fact Albustix with routine testing can measure levels greater than 0.1 g/l of urine.

Metabolic control does not influence the course of nephropathy once clinical proteinuria has started.However microalbuminuria has been shown to be reversible¹⁶ with excellent control.Microalbuminuria is defined as a level of protein in the urine of 0.01-0.05 g/24 hours. Albustix is unable to detect this concentration of protein, but there are methods, which allow its measurement i.e. radio immunoassay, ELISA.

It would be quite reasonable to suggest that all diabetics should have their urine tested at least annually to determine whether they have microalbuminuria. It should be noted that despite proteinuria, renal function can remain normal in these patients for several years. Thus the measuring of urinary albumin at such low levels allows the clinician to identify those patients at risk of developing diabetic nephropathy. When renal function does start to decline, it does so progressively, without remission and

the rate of progression of nephropathy is best followed by serum creatinine concentrations. In fact the rate at which the disease is advancing can be estimated if the inverse of the creatinine values is plotted against time, a straight line is observed above a concentration of 3 mg/dl ($>200\text{mmol/l}$), the slope indicates whether the disease is advancing rapidly or slowly. Serum urea values can also be used but their predictive value is less.

b) Hyperlipidaemias

These may be divided into primary and secondary types, both being relevant to diabetes mellitus. In the primary hyperlipidaemias type II, IV and V, there is an associated carbohydrate intolerance but in the context of diabetes mellitus we are more concerned with the secondary hyperlipidaemias secondary to diabetes mellitus and these can be divided into three categories¹⁷.

1. Chronic poor control can produce massive over-secretion of triglycerides from the liver, this results in increased levels of VLDL in the periphery and aggravates the diabetic state by causing insulin resistance and a raised level of chylomicrons (due to reduced activity of lipoprotein lipase).

2. Acute ketoacidosis leads to a raised VLDL but chylomicrons are normal. Ketone bodies will circulate as a result of increased lipolysis.

3. Obesity is associated with type II diabetes. Raised levels of VLDL are found in these patients which can persist even when the diabetes is adequately controlled.

In addition it should be noted that HDL is low in diabetes mellitus and one should remember that in diabetics over the age of 50 years, the incidence of ischaemic heart disease is increased two or three fold. Therefore fasting levels of triglycerides and cholesterol should be monitored in diabetics.

d) C-peptide

C-peptide is formed during the conversion of pro-insulin into insulin in the granules of the beta cells of the islets of Langerhans. Radioimmunoassay and ELISA may be used to measure its level in plasma. The C-peptide remains in the beta granules alongside the insulin molecule from which it is released into the circulation simultaneously, molecule per molecule, whenever insulin secretion is stimulated. Since C-peptide is not appreciably broken down by passage through the liver it is an excellent measure of endogenous insulin secretion in the diabetic.

Thus the estimation of C-peptide is used in the diabetic for assessment of beta cell reserve, in other words to see if there is any residual function in patients with type I diabetes. It has been shown that in early diabetics especially children higher C-peptide values are found in patients with the best diabetic control.

In patients with secondary diabetes, (e.g. secondary to chronic pancreatitis) C-peptide concentration can be used as an index of beta cell reserve^{17,18}. In children, urinary C-peptide can be used to determine the beta cell reserve in preference to plasma levels.

e) Insulin Antibodies

It is reasoned that the presence of insulin antibodies may exert a favourable effect on metabolic control in type I and type II diabetics on insulin. The insulin antibodies act as a reservoir and bind and release insulin according to the reversible equilibrium between free and bound pools of the hormone.

"Brittle" diabetics, patients who have rapid swings of blood glucose may be deficient in insulin antibodies and therefore have no reservoir of insulin. So it may be useful to determine the level of insulin antibodies in some patients so that they may be managed more efficiently.

4. Hyperglycaemic Emergencies

Hyperglycaemia with dehydration and with or without acidosis is a medical emergency. Diabetic ketoacidosis is the most common form while the less frequently encountered hyperosmolar non-ketotic coma and lactic acidosis carry a high mortality.

Diabetic ketoacidosis

Diabetic ketoacidosis is a risk throughout the life of an insulin dependent diabetic. It may be defined as severe, uncontrolled diabetes requiring emergency treatment with intravenous fluids and insulin and

presenting with a total ketone body concentration in excess of 5 mmol/l¹⁹.

The diagnosis is based on the clinical presentation of dehydration and acidosis as ketone bodies are only measured retrospectively. It should be noted here that while the usual presentation is with accompanying hyperglycaemia, diabetic ketoacidosis may present with only minor elevations of blood glucose.

Patients usually present with history of thirst, polyuria, nausea and vomiting and on examination are dehydrated, with Kussmaul respiration. Only 20% or so of the patients are comatose.

On urine testing ketonuria and glycosuria are present and the diagnosis should be rapidly confirmed by blood glucose and blood gases. Base line measurements of electrolyte concentrations are essential as they determine the pattern of rehydration and electrolyte replacement.

Sodium

Rehydration is usually undertaken with saline (150mmol/l) 0.9% but may be changed if plasma sodium exceeds 150mmol/l. Plasma sodium may be low, normal or high depending on the relative loss of water or sodium. Sometimes the serum may be grossly lipaemic at presentation leading to pseudohyponatraemia with the common measuring methods, therefore plasma sodium may rise rapidly with rehydration and clearance of lipaemia with insulin. In these situations alternative saline solutions may be used as 75mmol/l (0.45%) or even 30mmol/l (0.18%). Both of these solutions should be used knowing that they carry the risk of haemolysis if used in excess.

Potassium

It is most important to avoid both hypokalaemia and hyperkalaemia during treatment, as both have serious possibly fatal consequences as regards cardiac arrhythmias. At presentation potassium may be low, normal or high. During treatment the main risk is from hypokalaemia. Plasma potassium levels will fall with rehydration and this action is more pronounced if alkali is used, also insulin administration will cause the cells to take up potassium so increasing the possibility of hypokalaemia.

Insulin

Insulin is given in small and regular amounts, the objective being to achieve a circulating concentration that will inhibit hepatic glucose output, promote glucose utilization and therefore inhibit lipolysis, leading to a fall in hepatic ketogenesis and correction of acidosis. Rapid acting insulin is used and the subcutaneous route is avoided because of the danger of poor perfusion and absorption.

The use of alkali is not without risk, although in theory acidosis may impair myocardial contractility and slow the glycolytic rate so partial correction using sodium bicarbonate may be carried out. But it leads to increased potassium loss in the urine^{19,20}.

Phosphate

At presentation the levels of 2:3 diphosphoglycerate (DPG) are low, meaning that the delivery of oxygen to the peripheral tissues is less effective. The administration of phosphate raises the levels of 2:3 DPG, but careful monitoring of potassium levels is required.

So the role of the laboratory in the management of diabetic ketoacidosis is of paramount importance, not only does careful monitoring help in the management and reduce the mortality but at the time of diagnosis the laboratory contributes to the assessment of the severity by providing glucose, electrolytes acid-base data²⁰.

Hyperglycaemic Non-ketotic Diabetic Coma

This condition is diagnosed when hyperglycaemia is accompanied by a plasma osmolality of more than 340 mosmol/kg with no significant acidosis. Mild ketonuria may be present.

Management is similar to that for ketoacidosis involving rehydration, insulin and electrolytes so therefore the demands on the laboratory are also similar. It should be remembered that the mortality is higher than in ketoacidosis.

Lactic Acidosis

Is rare in diabetics but they are not immune to lactic acidosis from hypovolaemic or septic shock. An

additional risk for diabetics is from biguanide treatment.

In conclusion the role of the Laboratory is to provide services to the clinician which will be of use to them under the following headings:

1. Confirming the diagnosis of diabetes mellitus.
2. Monitoring and achieving of diabetic control.
3. Monitoring long-term complications of diabetes mellitus.
4. Diagnosis and management of hyperglycaemic emergencies.

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