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Review Article

URINE C-PEPTIDE TEST: A NOVEL NON-INVASIVE TECHNIQUE FOR DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH TYPE-1 DIABETES

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ABSTRACT

There is a worldwide prevalence of diabetes along with other disorders across the globe. Numerous techniques are used for its diagnosis and management depending upon the type of diabetes viz Type 1, Type 2 and its other types. C-peptide is a protein that is produced in the body along with insulin. First preproinsulin is secreted with an A-chain, C-peptide, a B-chain, and a signal sequence. The signal sequence is cut off, leaving proinsulin. Then the C-peptide is cut off, leaving the A-chain and B-chain to form insulin.C-Peptide measurement in the blood of diabetic patient is used for the diagnosis of patient with Type-1 diabetes. But because of its invasive nature and poor patient compliance, a novel diagnostic test viz. Urine C-Peptide test is used. Urine C-peptide gives the estimation of C-Peptides in urine which has a good correlation with C-Peptides in the blood. Further work is needed to examine the optimum measurement and potential clinical uses of this novel test.

Keywords: C-peptide, Novel diagnostic test, Non-invasive diagnosic test,Urine C-Peptide test.

INTRODUCTION

The term "diabetes mellitus" describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs (WHO 1999). The estimated prevalence of diabetes among adults was 7.4% in 1995; this is expected to rise to 9% in 2025[1].

There are two main types of diabetes

Type-1 diabetes (T1B) usually develops in childhood and adolescence and patients require lifelong insulin injections for survival.

Type 2 diabetes (T2B) usually develops in adulthood and is related to obesity, lack of physical activity, and unhealthy diets.

Other categories of diabetes includes gestational diabetes (a state of hyperglycaemia which develops during pregnancy) and "other" rarer causes (genetic syndromes, acquired processes such as pancreatitis, diseases such as cystic fibrosis, exposure to certain drugs, viruses, and unknown causes

In diabetes, hyperglycemia causes symptoms of increased thirst, increased urination, increased hunger and weight loss. However, in the long-term, it causes damage to eyes (leading to blindness), kidneys (leading to renal failure), and nerves (leading to impotence and foot disorders/ possibly amputation). As well, it increases the risk of heart disease, stroke, and insufficiency in blood flow to legs. Studies have shown that good metabolic control prevents or delays these complications.

Thus, the primary goal of treatment is to bring the elevated blood sugars down to a normal range, both to improve symptoms of diabetes as well as to prevent or delay diabetic complications. Achieving this goal requires a comprehensive, coordinated, patient-centred

approach on the part of the health care system. [1]

Type 1 diabetes (T1D)

It is also known as Insulin-Dependent Diabetes Mellitus (IDDM). It is characterized by hyperglycemia due to an absolute deficiency of the insulin hormone produced by the pancreas.

It is usually (but not always) caused by autoimmune destruction of the beta cells of the pancreas, with the presence of certain antibodies in blood.

In this condition, patients require lifelong insulin injections for survival. It usually develops in children and adolescents (although can occur later in life). It may be present with severe symptoms such as coma or ketoacidosis. The patients are at increased risk of developing micro-vascular and macro-vascular complications. It often causes loss of consciousness or severe nausea/vomiting (ketoacidosis) or coma. Ketoacidosis more common in T1D than in T2D [2].

Diagnosis of Type 1 Diabetes[3]

The symptoms of type-1 diabetes begin to appear quickly once the pancreas shuts down its production of insulin. Hence, most people are diagnosed within a short period of time from when the symptoms begin. In some cases it may take longer. The tests used for diagnosing type 1 diabetes for any particular person depends on the situation and the doctor's preference

Tests used for diagnosing Type-1 diabetes

Diagnosis of type-1 diabetes can be done in 2 ways as follows:

1) **Measuring the blood glucose level [3, 4]: In these tests, generally the glucose levels in the blood are assessed and correlated to the progress of the disease in the patient.**

These tests are

1) Fasting Blood Glucose (FBG) Test
2) Oral Glucose Tolerance Test (OGTT)
3) Hemoglobin A1c Test

Limitations

1) It does not help us in distinguishing type-1 diabetes from type-2 diabetes since increase in the blood glucose level is observed in both the types of diabetes.
2) It does not give a reliable parameter for assessing the status of the disease in-vivo.

2) **Measuring the Parameters which have relevance to insulin production from the pancreas such as C-peptides in the blood**[3, 4]

C-peptide is a protein that is produced in the body along with insulin. First preproinsulin is secreted with an A-chain, C-peptide, a B-chain, and a signal sequence. The signal sequence is cut off, leaving proinsulin. Then the C-peptide is cut off, leaving the A-chain and B-chain to form insulin.

C-Peptide Test[5]

A C-peptide test measures the level of this peptide in the blood. It is generally found in amounts equal to insulin because insulin and C-peptide are linked when first made by the pancreas. Insulin helps the body use and control the amount of sugar (glucose) in the blood. Insulin allows glucose to enter body cells where it is used for energy. The level of C-peptide in the blood can show how much insulin is being made by the pancreas. C-peptide does not affect the blood sugar level in the body.

A C-peptide test can be done when diabetes has just been found and it is not clear whether type-1 diabetes or type-2 diabetes is present. A person whose pancreas does not make any insulin (type 1 diabetes) has a low level of insulin and C-peptide. A person with type 2 diabetes has a normal or high level of C-peptide.

Newly diagnosed diabetes patients often get their C-peptide levels measured as a means of distinguishing type-1 diabetes and type-2 diabetes. C-peptide levels are measured instead of insulin levels because insulin concentration in the portal vein ranges from two to ten times higher than in the peripheral circulation. The liver extracts about half the insulin reaching it in the plasma, but this varies with the nutritional state. The pancreas of patients with type-1 diabetes is unable to produce insulin, and, therefore, they will usually have a decreased level of C-peptide, whereas C-peptide levels in type 2 patients are normal or higher than normal.

An insulinoma causes the pancreas to release too much insulin, which causes blood sugar levels to drop (hypoglycemia). A person with an insulinoma will have a high level of C-peptide in the blood.

Preparation for C-Peptide test[6-8]

Some important measures which are to be taken for preparation of the patient for the C-Peptide test are as follows:

1. The patient is asked to stop eating and drinking for 8 hours before having this blood test.
2. Insulin and some oral medicines used to treat type 2 diabetes can change the test results. Hence, these medicines should be stopped before your blood test.

Procedure for C-Peptide test [6-8]

The procedure for carrying out the C-Peptide test is as follows

1. An elastic band is wrapped around the upper arm to stop the flow of blood. This makes the veins below the band larger so it is easier to put a needle into the vein.
2. The needle site is then cleaned with an alcohol.
3. The needle is then put into the vein. More than one needle stick may be needed.
4. A tube is then attached to the needle to fill it with blood.
5. The band is then removed from the arm when enough blood is collected.
6. A gauze pad or cotton ball is put over the needle site as the needle is removed.
7. The pressure is then applied to the site and then a bandage is been put at the needle site.

Risks for C-peptide test[6-8]

There are few risks which are to be taken care off while performing this C-peptide test. Some of them are as follows:

Though there is a very little chance of problem from having blood sample taken from a vein, a small bruise may be formed at the site. The chance of bruising can be lowered by keeping pressure on the site for several minutes. In rare cases, the vein may become swollen after the blood sample is taken. This problem is called phlebitis. A warm compress can be used several times a day to treat this. On-going bleeding can be a problem for people with bleeding disorders. Aspirin, warfarin and other blood-thinning medicines can make bleeding more likely.

Parameters for the C-peptide test [6-8]

A C-peptide test measures the level of this C-peptide in the body whose normal value in the blood is significant for diagnosis of Type 1 diabetes.

Normal value

The normal values listed here-called a reference range. The level of C-peptide in the blood must be read with the results of a blood glucose test. Both these test are done at the same time.

C-peptide level (Fasting): 0.51-2.72 nanograms per milliliter (ng/ml) or 0.17-0.90 nanomoles per liter (nmol/L)

High values

- High levels of both C-peptide and blood glucose are found in people with type 2 diabetes or insulin resistance (such as from Cushing's syndrome).
- A high level of C-peptide with a low blood glucose level may mean that an insulin-producing tumour of the pancreas (insulinoma) is present or that the use of certain medicines such as sulfonylureas (for example, glyburide) is causing the high level.
- If C-peptide levels are high after an insulinoma is taken out, it may mean that the tumour has returned or that the tumour has spread to other parts of the body (metastasized).

Low values

- Low levels of both C-peptide and blood glucose are found in liver disease, a severe infection, Addison's disease, or insulin therapy.
- A low level of C-peptide with a high blood glucose level is found in people with type 1 diabetes.
- Complete removal of the pancreas (pancreatectomy) causes a C-peptide level so low it cannot be measured. The blood glucose level will be high, and insulin will be needed in order for the person to survive.

Parameters which affect the C-Peptide Test[6-8].

The main factors which affect the reproducibility of the results include:

1. **Concomitant medications**

Taking medicines, such as insulin, or sulfonylureas simultaneously may affect the results of this test.

2. **Kidney failure**

Both insulin and C-peptide are removed from the body by the kidneys. C-peptide levels may be high in a person with kidney failure.

3. **Obesity**

More insulin is made in obese people and can cause high levels of C-peptide.

Precautions to be taken during the C-Peptide test[6-8]

1. A C-peptide test must be done at the same time as a blood glucose test.
2. A person with new type-2 diabetes often has a normal or high level of C-peptide in the blood. Over time, a person with type 2

diabetes may develop a low level of C-peptide.

3. A C-peptide stimulation test may be done to help tell the difference between the two types of diabetes. During a C-peptide stimulation test, a blood sample is taken to measure C-peptide. Then a shot of a hormone to increase blood sugar (glucagon) is given into a vein in the arm. Another blood sample is taken. In people with type 1 diabetes, C-peptide levels will be low because the pancreas cannot make any insulin in response to the glucagon. In people with type 2 diabetes, C-peptide levels will be higher than the first blood test because the pancreas is making more insulin in response to the glucagon.

Advantages of C-peptide test

1) It helps in distinguishing the type-1 from type-2 diabetes and prediction of treatment response. It could have important implications for management decisions; for example, insulin withdrawal or the addition of agents that rely on endogenous insulin secretion for their action, such as sulphonylureas, glucagon-like peptide-1 agonists or dipeptide peptidase-4 inhibitors [9].

2) It also helps to find the cause of hypoglycemia, such as excessive use of medicine to treat diabetes or a non-cancerous growth (tumour) in the pancreas (insulinoma) [10].

3) It is also used to check whether a tumor of the pancreas (insulinoma) was completely removed [11, 12].

Limitations of C-Peptide test[13,14]

Though it is quite useful for assessing the diagnosis and prognosis of the disease, it has some limitations related to patient compliance. Its main limitation is that it requires withdrawal of the blood samples every time from the patients for assessing the level of C-peptide in the blood which is quite cumbersome for some patients. Hence, to remove the limitation of this proven test a novel technique for detection of C-peptide in the urine has been established which would be very beneficial for the patients of type-1 diabetes.

Novel diagnostic test for diagnosis and management of type-1 diabetes[15]

Urine C-Peptide measurement test

It involves measuring the level of C-peptides in the urine rather than measuring it in the blood. It has been estimated that C-peptide is renally metabolized and approximately 5-10% of pancreatic C-peptide is excreted unchanged in the urine[16-18]. The 24-hrs urine collections have been shown to correlate well with serum C-peptide measurements. However, they are cumbersome and prone to incomplete urine collection[18]. Correcting urinary C-peptide for urinary creatinine adjusts for differences in urinary dilution and enables assessment of insulin secretion using a spot sample. Post-meal urine C-peptide creatinine ratio has been shown to have similar sensitivity and specificity to glucagon-stimulated serum C-peptide in classifying diabetes by insulin requirement.[20]

Urine Sample Collection

In this test, patients collect their urine sample after a standard

Breakfast .They are asked to fully empty their bladder immediately pre-meal and collect urine in containers with boric acid preservative 2 h after meal completion since Urine C-peptide collected in boric acid has recently been shown to be stable for 72 h at room temperature with no decline in C-peptide levels over this time[20]. Samples were returned by post or by hand. All samples were received within 72hrs of collection and were then frozen at -80^{0}c for subsequent analysis. Participants took their normal insulin and medication on the days of sample collection, except where a reduction in insulin before the provided breakfast was deemed necessary to avoid hypoglycemia.

Case study[15,20]

Jillian, 35 has benefited from the home urine test. She was diagnosed with diabetes aged 19 and put on insulin injections. The urine test identified that she is still making her own insulin 14 years after being diagnosed and a DNA test confirmed that she has a genetic type of diabetes. After 14 years of insulin treatment, Jillian is now off her insulin injections.

Advantages[20]

Urine C-Peptide test -

a. Provides a Simple practical measurement of insulin secretion for use in routine clinical practice
b. Is similar in cost to serum C-peptide test as it mainly uses the same reagents
c. Is a Non-invasive technique
d. Is Sensitive in nature and
e. Is Specific for long-duration type-1 diabetes.

Limitation[21, 22]

a. Though it has several advantages over other test but it is not without limitation. Its

main limitation is that C-peptide metabolism largely occurs in the kidney (20) hence the utility of Urine C-Peptide Test in renal impairment cases of diabetes is not predictable and still remains to be established.

b. Although they are well correlated, it is likely that the urine C-peptide creatinine ratio after individuals' own home meals will be less reproducible than measurement after a fixed stimulus. The absolute values seen are lower with home meals than with the mixed-meal tolerance test, suggesting B-cells are not maximally stimulated and therefore variations in meal content will affect urine C-peptide creatinine ratio levels and reproducibility

RESULTS AND DISCUSSIONS

A strong correlation between both fasting and stimulated serum C-peptide and urine C-peptide-creatinine ratio in late-onset, insulin-treated diabetes provides a sensitive and specific test for marked insulin deficiency[21].

The practical advantages of home urine C-peptide creatinine ratio collection over the serum C-peptide are substantial and could enable urine C-peptide creatinine ratio to become a useful clinical measure. Measurement could be performed in primary care rather than being limited to areas with immediate clinical access to a laboratory and will be significantly less time consuming for clinical staff[21,22].

CONCLUSION

Urine C-peptide creatinine ratio is well correlated with serum C-peptide and may provide a practical alternative measure to detect insulin deficiency for use in routine clinical practice. In conclusion, the study shows that home urine C-peptide determination can provide a practical alternative to serum C-peptide in detecting severe insulin deficiency amongst adults with late-onset, insulin-treated Type-1 diabetes. Further work is needed to examine the optimum measurement and potential clinical uses of this novel test.

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