DIAGNOSTIC BIOMARKERS FOR DIABETES: A REVIEW

ABSTRACT:

INTRODUCTION: In the year 2021, about 537 million adults between the age group of 20-79 years are living with diabetes. The expected number of individuals living with diabetes will be 643 million by 2030 and 783 million by 2045 (1). The diagnosis of diabetes and prediabetes with the help of various biomarkers, at right time with accuracy can help in improving the scenario in which the diagnostic biomarkers play an important role. Prediabetes is an intermediate condition of hyperglycemia characterized by impaired fasting glucose levels and impaired glucose tolerance (2). Biomarkers can be defined as “biological molecules that represent health or disease state” (3).

DISCUSSION: The conventional diagnostic biomarkers of diabetes include fasting and postprandial blood glucose levels, HbA1c, fructosamine, glycated albumin, etc. (4). These biomarkers have limitations like moderate sensitivity and specificity. As diabetes is mostly associated with other comorbidities, these biomarkers become inaccurate in several clinical conditions (5,6). Recently, LAMA2, MLL4, and PLXDC2 are found to be novel and reliable serum protein markers for pre-diabetic diagnosis in humans (7).

CONCLUSION: Therefore, there is a consistent exploration of more accurate and novel diagnostic biomarkers. The exploratory study of novel diagnostic biomarkers can help in the accurate diagnosis of prediabetes as well as diabetes with other comorbidities. Another approach for better diagnosis is to combine several biomarkers. In the future, more reliable diagnostic biomarkers and their combinations can be optimized for the diagnosis of prediabetes as well as diabetes.

Keywords: Diabetes, Biomarkers, Diagnosis, HbA1C, OGTT, Inflammatory biomarkers, Noval Biomarkers.

INTRODUCTION:

“Diabetes is a metabolic disorder characterized by hyperglycemia due to insulin insufficiency and insulin resistance or both. It accompanies other comorbidities like obesity, lipotoxicity, kidney disorders, etc”. (8). The American Diabetes Association has classified diabetes as -

1. Type 1 diabetes, (autoimmune destruction of beta cells in the pancreas leading to loss of synthesis of insulin),
2. Type 2 diabetes (progressive insufficient insulin production along with insulin resistance),
3. Specific types of diabetes due to other causes, like - monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young), exocrine pancreas disease such as cystic fibrosis and pancreatitis, and drug- or chemical-induced diabetes, like in the case of glucocorticoid use, in HIV/AIDS treatment, or after organ transplantation),
4. Gestational diabetes mellitus (due to altered metabolism in pregnancy) (9).

"The treatment strategy for type 1 diabetes includes insulin therapy and administration of hypoglycaemic agents. For the treatment of type 2 diabetes and other types of diabetes, the current pharmacotherapy includes the use of hypoglycaemic agents" (10).

"Type 1 diabetes is characterized by the autoimmune destruction of beta Langerhans cells of the pancreas, which leads to loss of insulin production. The reduced insulin levels cause the inability of absorbing the blood glucose in cells, which leads to hyperglycemia. Thus, type 1 diabetes is related to insulin insufficiency. In type 2 diabetes, peripheral insulin resistance is observed with progressive loss of insulin production, leading to hyperglycemia" (11).

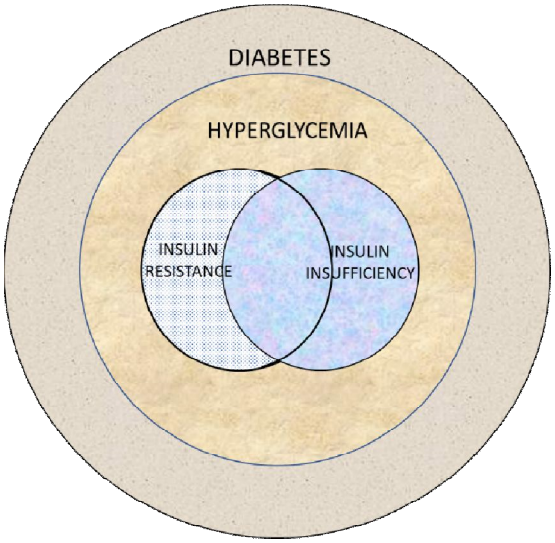

Fig 1: Diabetes scenario

“Prediabetes is a health state where the blood sugar levels are higher than normal but not higher enough to be classified as a type 2 diabetic condition” (12). The American diabetes association defined the fasting blood sugar levels for prediabetes as 100-125 mg/dl and for diabetes, it's above 125mg/dl (1). These values might be appropriate in the context of America but for the countries like India, the values do not hold up well.

BIOMARKERS FOR THE DIAGNOSIS OF DIABETES:

Biomarkers, or biological markers, are the biological molecules that signify the state of health or disease in an individual. To assess the disease condition, there are certain molecules present in the body, and their presence, absence, or abundance is checked to diagnose a disease. The diagnosis of diabetes and prediabetes with the help of various biomarkers, at right time with accuracy can help in improving the scenario in which the diagnostic biomarkers play an important role. Prediabetes is an intermediate condition of hyperglycemia characterized by impaired fasting glucose levels and impaired glucose tolerance. The conventional diagnostic biomarkers of diabetes include fasting and postprandial blood glucose levels, HbA1c, fructosamine, glycated albumin, etc. These biomarkers have limitations like moderate sensitivity and specificity. As diabetes is mostly associated with other comorbidities, these biomarkers become inaccurate in several clinical conditions. Recently, LAMA2, MLL4, and PLXDC2 are found to be novel and reliable serum protein markers for pre-diabetic diagnosis in humans.

Lifestyle and pharmacological interventions at the prediabetes stage can prevent the progression of diabetes. But the point is the detection and risk management of a prediabetic state. Several authorities like International Diabetes Association(IDA), American Diabetes Association(ADA), World Health Organisation, etc. are working in this direction but a concrete outcome is still far away.

CLASSIFICATION OF DIAGNOSTIC BIOMARKERS FOR DIABETES:

Biomarkers for diagnosis of diabetes can be majorly classified as –

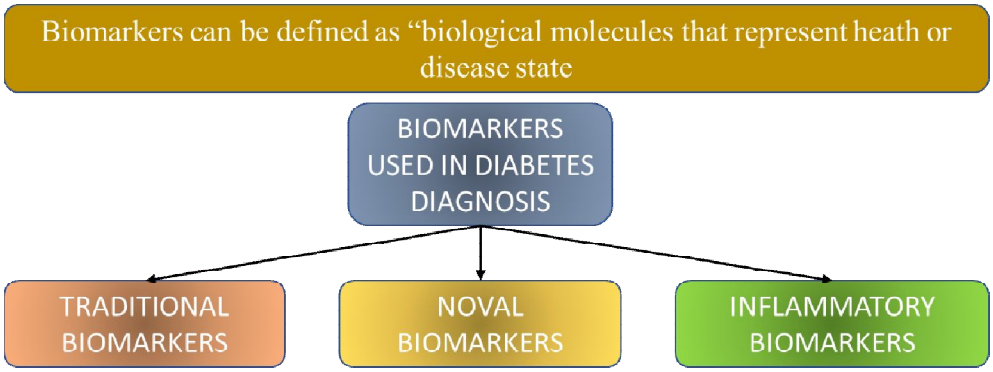

Fig 2: Classification of diagnostic biomarkers for diabetes

TRADITIONAL BIOMARKERS:

Traditional biomarkers are used most commonly and are conventional, e.g. OGTT, Hb1Ac, etc. The following table discusses the ADA-recommended biomarkers –

Biomarkers Recommended by American Diabetes Association			
Biomarker	Standard range	Advantage	Disadvantage
Fasting Blood Glucose levels	Normal : <100mg/dl Prediabetes : 100-125mg/dl Diabetes : >126mg/dl	Convenient, Direct	Fasting condition is neccessary
HbA1c	Normal : <5.7% Prediabetes : 5.7-6.4% Diabetes : >6.4%	More reliable biomarker of chronic glycemia, less day-to-day fluctuation.	Moderate sensitivity, no consideration of BMI, age, or ethnicity, etc., hemoglobin variants and life span of RBC's affect the value.
OGTT (Oral Glucose Tolerance Test)	Normal : <140mg/dl Prediabetes : 140-199mg/dl Diabetes : >200mg/dl	Strong indicator of Insulin secretion and resistance, Indicator of Type 2 diabetes	Variable, invasive, time-consuming

Table 1: Biomarkers recommended by ADA.

HbA1c:

It is a biomarker that is frequently used to identify people with diabetes and prediabetes. When glucose binds to the amino-terminal group of the haemoglobin subunit, HbA1c is created. Glucose levels across time rather than only at one time point are shown by HbA1c. Increased morbidity and death are linked to higher HbA1c values. A level of HbA1c less than 6.5% (48 mmol/mol) is linked to retinopathy. Fasting plasma glucose was not as significantly

connected with retinopathy as HbA1c was (FPG). As a result, HbA1c rather than FPG may be a better indicator of microvascular problems. (13-19).

Oral glucose tolerance test (OGTT)

“Type 1 diabetes, type 2 diabetes, and gestational diabetes mellitus can all be identified with a glucose tolerance test. It is a blood test that requires drawing several blood samples over the course of two hours, on average. Compared to HbA1c, OGTT has a stronger correlation with IR and secretion” (20). A BC-shielded IV catheter can be inserted, or several phlebotomy draws can be used to do the test. It is necessary to collect the fasting sample and record the time. The patient should next take the appropriate dosage of glucose (determined by weight, up to 75 grammes) over the course of no more than 5 minutes. (21-25).

Glycated albumin (GA)

A non-enzymatic glycation process of serum albumin results in GA, a ketoamine. Because albumin has a half-life of roughly 15 days, glycated albumin (GA) rises in the presence of hyperglycemia and reflects mean glycemia over a period of two to three weeks. Before starting or switching drugs, GA can be used to assess the efficacy of treatment because it changes more quickly and more significantly than HbA1c. Patients with anaemia or hemoglobinopathies, for whom the clinically determined HbA1c level may be off, can also benefit from GA. Compared to HbA1c alone, the GA and HbA1c combination had a higher sensitivity for predicting prediabetes. For individuals with renal failure, hemolytic anaemia, and those receiving blood transfusions, GA is a better indicator of glycemic control than HbA1c. In clinical disorders that cause protein loss, such as nephrotic syndrome, liver disease, and thyroid illness, GA is preferable to FA. (26-35).

Fructosamine (FA)

As a substitute glycemic marker for diabetes screening, fructosamine (FA) has been employed.

may possibly helpful for identifying prediabetes. The glycosylation of total serum proteins, principally albumin, results in the formation of FA, a ketoamine. In conditions of high glucose concentrations, FA rises. it displays the typical blood glucose levels during the last 1-4 weeks,

It may serve as a helpful clinical marker for glucose control and short-term glycemic variation. FA is particularly helpful in circumstances that compromise the consistency of

haemoglobin. It doesn't call for fasting. In clinical situations when HbA1c may not be reliable, FA may be a useful supplementary measure. FA is particularly helpful for ailments that impact the level of haemoglobin or the pace of erythrocyte turnover. FA is practical, easy, and affordable (34-40).

GLYCATED ALBUMIN(GA):

Albumin glycosylation as determined by the proportion of GA to total albumin. For individuals with renal failure, hemolytic anaemia, and those receiving blood transfusions, GA is a better indicator of glycemic control than HbA1c. In clinical circumstances such nephrotic syndrome, liver disease, and thyroid illness that cause protein loss, GA is recommended to FA. In people with elevated BMI, body fat mass, and visceral adiposity, GA may be unnaturally low.

Although it has many benefits, it also has some downsides, such as inaccurate results when albumin turnover changes and falsely low levels in the case of obesity (32-40).

Traditional biomarker				
Biomarker	Mechanism of action	Advantages	Disadvantages	Association with dysglycemia
1. OGTT	Measures fasting and 2-hour plasma glucose levels	OGTT is more strongly correlated with IR and secretion than HbA1c. OGTT provides important information with regard to risk that HbA1c or FBG cannot.	OGTT is variable, invasive, and time consuming It is inconvenient because it requires fasting and shows day-to-day perturbation during periods of stress and illness.	Elevated FBG and 2-hour levels are associated with prediabetes and diabetes
2. HbA1c	HbA1c is formed when glucose attaches to the amino-terminal group of the β subunit of hemoglobin	Increased HbA1c levels are associated with increased morbidity and mortality. More reliable biomarker of chronic glycemia HbA1c correlates with greater convenience, greater pre-analytical stability, and less dayto-day perturbation during periods of stress and illness.	HbA1c has moderate sensitivity in diagnosing diabetes when compared to OGTT and FBG. No consensus which cut-off points for HbA1c would be most sensitive. HbA1c threshold for prediabetes does not consider ethnicity, BMI, and age, all of which may significantly alter HbA1c levels. HbA1c is not always a reliable measure of average circulating glucose levels. Changes in the production rate or circulating life span of red blood cells affect HbA1c levels, as well as hemoglobin variants such as HbS, HbC, HbD, and HbE.	HbA1c is a reflection of chronic glycemia.
3. FA	FA is a ketoamine created by glycosylation of total serum proteins, primarily albumin	FA reflects average blood glucose concentration over the previous weeks. FA is especially beneficial in conditions that affect hemoglobin status or rate of erythrocyte turnover. FA is cost-effective, simple, and convenient, as it does not require fasting.	FA has higher within-subject variation and falsely low levels in conditions leading to rapid albumin turnover. Not all studies have found that mean serum FA levels are useful for prediabetes screening.	FA increases in states of high glucose concentrations.
4. GA	Glycosylation of albumin and measured by the ratio of GA to total albumin	GA is a superior index of glycemic control than HbA1c in patients with renal failure, hemolytic anemia, and those receiving blood transfusions. GA is preferred over FA in clinical conditions that result in protein loss such as nephrotic syndrome, liver disease, and thyroid disease. GA may be artificially low in individuals with increased BMI, body fat mass, and visceral adiposity.	Inaccurate when there are changes in albumin turnover Falsely lower levels in obesity.	Serum GA is associated with prediabetes and diabetes.
5. 1, 5 AG	1,5 AG is a dietary monosaccharide. Plasma	1,5 AG is a useful biomarker as it reflects glucose levels within the past 10–14 days It is stable, replicable, and less costly compared to other glycemic diagnostic tests.	Plasma 1,5 AG levels can change based on dietary habits, sex, and race64,65 Levels are also affected by renal hemodynamics or treatment with SGLT2 inhibitors.	Plasma 1,5 AG levels are lowered in subjects with prediabetes and diabetes compared with subjects with normoglycemia

Table 2 : Traditional biomarkers used for diabetes diagnosis.

Novel Biomarkers:

Pancreastatin:

“Pancreastatin is a proteolytic cleavage product of Chromogranin A, which has a highly significant role in several pathological conditions like cancers, diabetes, etc. Patients with

diabetes are said to have higher increased PST levels (specifically in non-insulin-dependent Diabetes Mellitus: NIDDM). The PST increases glucagon release while inhibiting the first stage of glucose-induced insulin secretion. This increase causes peripheral insulin resistance, which ultimately results in diabetes. Pancreastatin levels may offer promising indicators for the diagnosis of diabetes based on this phenomena". (41-44).

Adiponectin:

"Based on clinical data, adiponectin can be regarded as a standalone predictor of diabetes. It is known to have anti-atherogenic, anti-inflammatory, and insulin-sensitizing characteristics and is generated from adipose tissue. Lower adiponectin levels are seen considerably before the prevalence of diabetes in many people, making it a viable biomarker for prediabetes. In diabetes preventive trials, lower adiponectin levels are linked to insulin resistance and obesity, while greater levels are linked to lifestyle intervention groups. Males and females can be distinguished by their adiponectin levels more clearly than each other. Adiponectin levels were directly correlated with insulin sensitivity and indirectly with insulin secretion using the hyper-insulinemic euglycemic clamp and intravenous glucose tolerance test" (45-47).

Fetuin-A

It is also known as alpha-2-HS-glycoprotein, and in humans, the AHSG gene is responsible for encoding it. It is a glycoprotein with a mass unit of 64 kDa that is mostly secreted by the liver. Through the inflammatory signalling cascade involving the toll-like receptor-4 (TLR-4) it has been discovered to support lipid-induced insulin resistance. Free fatty acids regulate fetuin-A levels, and fetuin-A promotes insulin resistance by blocking the IRS-1 and Akt complex in the insulin-dependent Akt pathway. T2DM is ultimately brought on by more gluconeogenesis brought on by G6Pase and PEPCK as well as tissue inflammation brought on by interleukins. Clinical investigations further support the assumption that fetuin-A plays a crucial function as a diagnostic marker for diabetes since it contributes to the development of insulin resistance (48-51).

miRNAs

Non-coding RNAs called miRNAs play a role in post-translational gene expression. They support a number of physiological processes, including cell death, differentiation, and

proliferation. Triglyceride levels and miR-193b and miR-192 play a key impact in prediabetes and diabetes.

“T2DM has been discovered to have higher levels of miRNAs such miR-9, miR-29a, miR-30d, miR-34a, miR-124a2, miR-146a, and miR-375. All of these alter the beta cells' physiological processes and impair insulin expression and secretion. Other miRNAs, such as miR-126, have been reported to decrease in people with diabetes”. (52-58).

Novel biomarkers	
Biomarker	**Association with dysglycemia and complication**
6. Adiponectin	Lower levels of adiponectin are associated with increased IR and obesity, while higher levels have been related to lifestyle intervention groups in diabetes prevention trials68 Adiponectin levels are inversely related to the risk of incident prediabetes, independent of ethnic or sex differences. Adiponectin levels were directly correlated with insulin sensitivity and indirectly correlated with insulin secretion.
7. FetA	FetA correlates with increased risk of developing T2DM and associated complications.
8. a-HB	IR, increased oxidative stress, and lipid oxidation may cause chronic shifts in glutathione synthesis leading to elevated α-HB levels88 α-HB was found to be significantly associated with IR independent of sex, age, BMI, and collection site. IR was associated with reductions in glycine and serine, which are upstream of α-KB
9. L-GPC	L-GPC is a negative predictor of T2DM progression.
10. Lp(a)	Lp(a) has an inverse relationship with prevalence of prediabetes and T2DM.
11. Triglycerides	Associated with β-cell dysfunction and reduced insulin secretion in subjects with prediabetes.
12. HDL	HDL-C promotes insulin secretion
	• Low HDL-C concentration may lead to progression from
	• Increased proportion of small HDL3 over HDL-C in subjects with
	• Decreased proportion of HDL-LpPLA2 in prediabetes.
13. Ceramide	Positively associated with prediabetes and T2DM.
14. Ferritin and transferrin	Elevated serum ferritin and transferrin saturation have been strongly associated with increased risk of prediabetes and diabetes. Iron has properties that contribute to IR such as production of highly active radical formation, damage to DNA and cell membrane integrity, β-cell oxidative stress leading to decreased insulin secretory capacity, and interference with glucose uptake in skeletal muscles and adipocytes. Moreover, catalytic iron stimulates the formation of reactive oxidative species, hepatic dysfunction, and β-cell apoptosis, all of which contribute to IR.
15. MBL -associated serine	MASP1 has been shown to positively correlate with prediabetes, diabetes, and CVD.
16. THBS1	THBS1 positively associated with:
	• Higher prediabetes prevalence
	• Increased IR
	• Increased 2-hour glucose
	• Adipose inflammation and metabolic dysregulation in obesity and type 2 diabetes
17. GPLD1	GPLD1 positively associated with:
	• Type 2 diabetes and prediabetes (less strongly)
	• MASP1, another novel prediabetes biomarker
	• HDLs in serum
18. Acylcarnitine	Elevated levels of acyl-carnitine found in individuals with prediabetes Associated with inflammation and IR.
19. miRNA	Many miRNAs have been found to be elevated in individuals with prediabetes. miR-192 and 193b associated with subjects with IFG and IGT; associated with high triglyceride levels and fatty liver index. Other miRNAs play a role in insulin production, secretion, and regulation.

Table 3: Novel biomarkers for diabetes diagnosis.

Inflammatory biomarkers:

CRP

The liver produces CRP, a pentameric protein that rises in reaction to inflammation. The impact of IL-6 on the gene that controls CRP transcription results in the production of CRP, an acute-phase reactant protein, in major part during the acute phase of an inflammatory or viral event. CRP has both pro- and anti-inflammatory properties. It assists in the detection and removal of invasive infections and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin. It can activate phagocytic cells as well as the traditional complement system via Fc receptors, making it easier to get rid of cellular waste, damaged or dead cells, and foreign infections. However, in auto-immune conditions such idiopathic thrombocytopenic purpura, when activated by autoantibodies expressing the phosphocholine arm, this might turn hazardous (ITP).

“90% of the time, CRP levels above 50 mg/dL are associated with bacterial infections. CRP has been used as a prognostic indicator in numerous studies, including those on hepatitis C, dengue fever, and malaria. On the other hand, mild elevations may or may not have clinical significance. It is highly recommended to use clinical correlation when evaluating CRP test findings” (59).

Prediabetes, a condition that occurs before diabetes, increases the risk of cardiovascular disease. Prediabetes is characterised by higher CRP levels in comparison to normal glucose tolerance, while diabetes is associated with high levels of C-reactive protein (CRP). Additionally, systemic inflammation may have an impact on the early impairment of glucose metabolism (60). While elevated glucose levels and insulin resistance are both major contributors to the inflammatory process, the basic processes causing elevated CRP levels in long-term type 1 and type 2 diabetes individuals may vary. Both type 1 and type 2 diabetes patients with persistently high blood glucose levels exhibit chronic inflammatory changes in their tissues, as indicated by elevated CRP levels. However, greater CRP levels have been found in people with near-normoglycemic pre-diabetic type 2 diabetes and metabolic syndrome, who are thought to have a high incidence of insulin resistance (61).

IL-6 -

A naturally occurring substance called interleukin-6 (IL-6) is involved in the development of B cells and inflammation. IL-6 is a pyrogen that induces fever in autoimmune, infectious, and non-infectious illnesses in addition to being an immunological protein. When the body

experiences either acute or long-term inflammation, IL-6 is released. This includes conditions such as injury, burns, cancer, and infection. This chemical stimulates the transcription of inflammatory gene products by interacting with the interleukin-6 receptor alpha. Numerous inflammatory chronic diseases have been connected to IL-6. It is also believed that interleukin-6 makes people more susceptible to diabetes (62).

“Increased levels of IL-6 have been linked to type 1 diabetes and may contribute to immunopathogenesis, which causes the immune system to kill islet cells in the pancreas. Contrarily, IL-6 appears to have physiologically significant roles in the islets, proving the cytokine's pleiotropic nature. With or without the extra adipose tissue that frequently goes along with diabetes, chronic inflammation is thought to play a role in insulin resistance in type 2 diabetes. The endothelium, as well as renal and brain tissue, are damaged by chronic hyperglycemia, which also develops in type 1 diabetes. The potential for anti-IL-6 intervention in type 1 and type 2 diabetes, on the other hand, is primarily related to the treatment of CVD and CKD, both of which diabetes is a major risk factor” (63).

IL-I RECEPTOR ANTAGONIST

“An IL-1 family member called the interleukin-1 receptor antagonist (IL-1Ra) binds to IL-1 receptors without eliciting an intracellular response. The synthesis of IL-1Ra is triggered by a variety of elements, such as adhering IgG, other cytokines, and bacterial or viral components. The tissue distribution of IL-1Ra in mice reveals that sIL-1Ra is primarily present in peripheral blood cells, lungs, spleen, and liver whereas icIL-1Ra is prevalent in skin” (64).

High glucose concentrations stimulate the synthesis of interleukin-1beta in human pancreatic beta cells, which impairs insulin secretion, inhibits cell proliferation, and causes cell death. Type 2 diabetes mellitus pancreatic islets exhibit decreased interleukin-1-receptor antagonist expression (65).

Inflammatory biomarker	
Biomarker	Association dysglycemia with complication
20. CRP	Associated with type 2 diabetes and IR Found to be associated with prediabetes
	CRP found more elevated in subjects who had prediabetes and IR than those with prediabetes but insulin sensitive.
21. IL-6	Associated with type 2 diabetes and IR131
22. WBCs	WBC count has been predictive of:
	• Worsening insulin action
	• Secretory function and T2DM
	• Coronary heart disease
	• Higher 1-hour post-load glucose level.

Table 4: Inflammatory biomarkers for diabetes diagnosis.

Conclusion:

When used in conjunction with a continuous pathophysiologic process, categorical or absolute definitions of dysglycemia may unintentionally underestimate individuals who are at risk for diabetes progression. Even within the so-called "normal range," gradually rising glucose levels happen rather late in the development of T2DM, when beta-cell function may already be compromised. Therefore, there is a critical need to find more accurate and sensitive biomarkers that can forecast the onset of dysglycemia at the earliest possible stage, when -cell function is still substantially more optimum and may be more amenable to lifestyle changes. In a clinical environment, combining biomarkers may improve the sensitivity and specificity of prediabetes and diabetes prediction. To determine the clinical value of biomarkers, more comparative studies will be needed. Additionally, genetic studies may be a good source to evolve some more noval and effective biomarkers for diabetes.

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