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Exploring Traditional and Emerging Biomarkers in Type 2 Diabetes Mellitus: A Comprehensive Overview of Diagnostic and Prognostic Tools

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Abstract
Type 2 Diabetes Mellitus is Among the most prevalent and globally widespread metabolic disorders. Though it has been associated with elderly people, recently the prevalence of younger individuals affected with T2DM has significantly increased. Currently, there is a broad range of diabetic biomarkers with those extensively used in clinical medicine such as HbA1c and those on ongoing research. Despite the competence of current diabetes biomarkers, they all display limitations in terms of accuracy and convenience, for this reason, there is a continuous urge to discover reliable, precise, and conventional biomarkers for early diagnosis and treatment of patients. This review encompasses a comprehensive background on both traditional as well as novel T2DM biomarkers therefore guides future research.
Key Words: advanced biomarkers, traditional biomarkers, type two diabetes, diagnosis.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM), which is known to be the most widespread diabetes type (more than 90%), is a disease mostly recognized in older individuals (Kahn et al., 2021). It is a metabolic, chronic condition (Bashir et al., 2024) associated with several factors such as environmental factors, age, lifestyle, diet, genetics, and others (Ortiz-Martínez et al., 2022). T2DM is characterized by the two primary pathological mechanisms of resistance to insulin and decreased pancreatic insulin production, particularly in skeletal and hepatic cells. Another significant factor in predicting the likelihood of T2DM is insulin resistance (IR) (Laakso, 2019).

The disease is linked to dysregulation of a wide range of biological pathways (Huang et al., 2019). The onset of diabetes is not sudden, rather it develops gradually and in most cases displays no symptoms for several years (Ortiz-Martínez et al., 2022). A biomarker, a trait that is suggested to be an indicator of pathological processes (Aghaei et al., 2020) can significantly contribute by working as a means to early diagnosis and risk of developing the disease (Huang et al., 2019)

Some issues with T2DM management require attention. Novel, more thorough biomarkers are required to achieve the best screening results, better diagnose individuals who are at risk of diabetes mellitus and its related complications, track the course of the disease, and evaluate the effectiveness of therapeutic measures, new biomarkers are required (Guay & Regazzi, 2013). In this review, we aim to encompass both traditional and newly emerging biomarkers along with their properties and use to provide an extensive overview of T2DM biomarkers.

Traditional Biomarkers

a. Blood glucose

A patient's blood sugar levels must be at or above a specific threshold to be diagnosed with diabetes. Four methods for diagnosing diabetes are listed by the American Diabetes Association (ADA), and these methods are also used to test patients for pre-diabetes. The methods are: First, the Fasting Plasma Glucose Test (FPG): this test defines "fasting" as abstaining from all food and liquids for a minimum of eight hours before the test; (3) the oral glucose tolerance test (OGTT): in this test, a patient takes a glucose syrup solution containing 75 g of glucose, after which a blood test is performed to measure 2-hour plasma glucose (PG); (4) random plasma glucose (PG) of more than or equal to 200 mg/dL or 11.1 mmol/L in patients who showed signs of hyperglycemia or hyperglycaemic crisis (Khan et al., 2019).

b. Hemoglobin A1c (HbA1c)

The most widely used diagnostic biomarker for diabetes and prediabetes is HbA1c. HbA1c is produced when glucose binds to the amino-terminal group of the hemoglobin β subunit. Rather than assessing glucose at a certain point in time, the HbA1c test assesses chronic glycemia. The current American Diabetes Association criteria for diabetes is HbA1c ≥6.5% (48 mmol/mol), while the norm for prediabetes is 5.7–6.4% (39–46 mmol/mol). Higher rates of morbidity and mortality are associated with elevated HbA1c levels. In the Norfolk prospective study, higher HbA1c levels were associated with higher rates of cardiovascular diseases (CVD), cancer, and all-cause death. Long-term follow-up studies have clearly shown a correlation between mean HbA1c and problems related to diabetes; in this case, retinopathy is linked to a level of ≥6.5% (48 mmol/mol). Moreover, there was a higher association

between retinopathy and HbA1c than between FPG (fasting plasma glucose). As a result, HbA1c rather than FPG might be a more reliable measure of microvascular issues. Compared to the FPG and the oral glucose tolerance test (OGTT), the HbA1c offers several benefits. It is more convenient because it doesn't require fasting, has better pre-analytical stability, and has less disruption to daily activities when under stress or ill. The HbA1c test is a crucial tool for lifestyle adjustment therapy since it details an individual's ongoing exposure to glucose. Data on the usefulness of HbA1c are inconsistent since it is less sensitive than OGTT and FPG in the diagnosis of diabetes. Because OGTT and IR are more strongly correlated than HbA1c, it makes sense to assume that a person's reaction to a high glucose consumption would more closely mirror their physiological response and insulin secretion and activity (Dorcely et al., 2017).

Some advantages for HbA1c were agreed upon as compared to plasma glucose levels obtained either two hours after an oral glucose load of 75 grams or during fasting. In particular, it was thought that HbA1c was less sensitive to biological variability, pre-analytic instability, prandial condition, and acute stress, and a more accurate and consistent test than glucose for measuring total glycemic exposure (International Expert Committee, 2009). Due to the potential financial constraints associated with HbA1c assays, the International Expert Committee has recommended glucose criteria as a means of diagnosing diabetes. It is difficult to conclude that HbA1c reliably represents mean blood glucose since biological factors such as age, race, genetics, and physiology affect the association between HbA1c and blood glucose. A multinational study with limitations, including the underrepresentation of some ethnic groups and the exclusion of some populations, found a strong association between HbA1c and estimated average glucose (eAG). Hemoglobin glycation is also influenced by changes in the lifespan of red blood cells and glucose gradients across RBC membranes. HbA1c is a commonly used biomarker for chronic glycemia in diabetes that responds to pharmaceutical and lifestyle therapies despite these problems. Numerous extensive investigations have exhibited its efficacy in the surveillance of diabetes and the prediction of complications (Lyons & Basu, 2012).

c. CD59

The CD59, which is a protein found on cell surfaces, stops cell lysis by preventing the development of membrane attack complexes. Pancreatic β-cells express CD59 at a high level, and this protein is essential for insulin release. Furthermore, in individuals with diabetes, glycation leads to its deactivation. Glycated CD59, or GCD59, is a biomarker for T2DM that was found to have a significantly higher expression level in people with diabetes. Furthermore, this biomarker and HbA1C have a positive correlation. This biomarker may be used as a possible biomarker to identify people with diabetes from healthy people, as it has a sensitivity and specificity of 93% and 100%, respectively (Ghosh et al., 2015).

Advanced Biomarkers

a. Growth-Differentiation Factor-15 (GDF-15)

This member of the TGF-β superfamily is known to be expressed in higher concentrations in certain cell types such as macrophages, endothelial cells, and adipocytes, it plays a key role in processes such as cell differentiation, development, and inflammatory responses. The levels of the expression of GDF-15 noticeably increase in people with insulin resistance and chronic kidney disease. Owing to these criteria, the concentration of serum GDF-15 is regarded as a significant diagnostic marker of T2DM. The increased levels are parallel with the increased levels of angiotensin-2 in patients with T2DM (Aghaei et al., 2020).

b. miRNAs

miRNAs are small molecules that are composed of 21 to 23 nucleotides, their significance lies in their ability to bind to their target genes and prevent their expression. They exhibit this effect on almost 30% of coding genes (Aghaei et al., 2020). They are released by cells to the extracellular matrix in different conditions to facilitate intercellular communication thus regulating vital biological processes such as new vessel formation, immunological processes, and tumor cell invasion. (Beltrami et al., 2015).

miRNAs are reported to be involved in diabetes-related complications such as retinopathy, and renal and cardiovascular diseases. Increased levels of miRNAs exhibited in different body fluids such as saliva, plasma, cerebrospinal fluids, and others grant them an important position as a biomarker in several diseases including T2DM (Aghaei et al., 2020) miRNAs are significant due to their ability to early T2DM diagnosis and their associated complications (Guay & Regazzi, 2013). miRNA is differentially expressed. While miR-375 lowers the likelihood of insulin resistance, other miRNAs such as miR-320a and miR-486-5p enhance it. Baseline levels of particular miRNAs (e.g., miR-15a, miR-126) accurately predicted the development of T2DM in the future. miRNA exhibits the potential of being a reliable biomarker of high specificity however to develop and confirm the efficacy of miRNAs further investigations must be carried out (Vasu et al., 2019).

c. Fructosamine (FA)

All stable ketamine products of the non-enzymatic glycation of the serum proteins like globulins, albumins, and others are referred to as fructosamine (Figure.1). Due to hyperglycemia in T2DM the FA, content in the serum rises. Because of the increased blood sugar level in T2DM, there is a rise in the

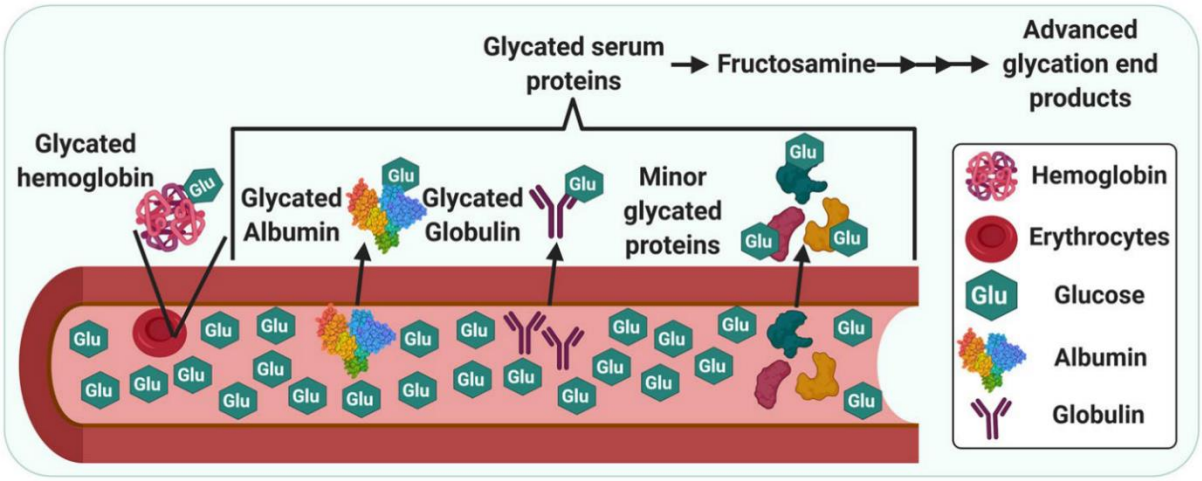

Figure1. The correlation between fructosamine, glycated proteins, and hyperglycemia is illustrated graphically (Dorcely et al., 2017).

concentration of FA in serum. Utilizing a glycemic marker, fructosamine (FA) may help differentiate between people without diabetes and those who have it. Because hemoglobin has a longer half-life in circulation, HbA1c indicates long-term glucose variations; in contrast, FA represents glucose levels during a period of two to three weeks. Furthermore, FA tests are less complex and more reasonably priced than HbA1c. The most popular techniques for evaluating FA are colorimetric-based techniques, which are quick, simple, affordable, and automatable. Research has demonstrated robust associations between FA and HbA1c in T2DM, exhibiting both high sensitivity and specificity in differentiating between those without diabetes and those with the condition. Additionally, fasting is not required for the FA test (Ortiz-Martínez et al., 2022). Furthermore, FA is associated with fast T2DM development and is linked to the rise of vascular diseases related to T2DM (International Expert Committee, 2009).

Rather than being used as a diagnostic or monitoring marker, FA appears to have more uses as a risk biomarker. When starting a new treatment regimen or monitoring glycemic status in individuals with poor glycemic control, FA as an intermediate marker is especially helpful. Future research should examine the connection between FA and the development of diabetic complications to evaluate the marker's potential as a risk signal in individuals who have already received a diagnosis (Ortiz-Martínez et al., 2022).

d. 1,5-Anhydroglucitol (1,5-AHG)

1,5-anhydroglucitol is a monosaccharide composed of six carbons, it is alternatively referred to as 1-deoxyglucose (Pitkänen, 1990). This biomarker is efficiently absorbed in the gastrointestinal tract, it comes primarily from the diet (where it is present in low amounts) and is physiologically stable. Its renal reabsorption and lack of a metabolic pathway for 1,5AHG breakdown also cause its tissue concentrations to attain steady state values. As a result, its concentrations are constant and associated with blood glucose in various bodily fluids. Urine excretion regulates the systemic 1,5AHG concentration. When compared to glucose at the sodium-linked transporters (SGLT)glucose for kidney absorption, 99.9% of 1,5AHG is absorbed in normoglycemic persons, meaning it is maintained in measurable amounts in blood and saliva. The extra glucose in hyperglycemia monopolizes the glucose transporters.

The short-term glycemic regulation, namely postprandial hyperglycemia, is shown by 1,5-Anhydroglucitol (1,5-AG). When blood glucose levels are beyond the renal threshold, 1,5-AG levels fall, in contrast to HbA1c, which gauges long-term glucose levels. 1,5-AG is therefore a helpful marker for glycemic management during the previous one to two weeks. It has a good connection with HbA1c and can distinguish between patients who have varying glycemic control but similar HbA1c levels. It is sensitive for the early identification of problems related to glucose metabolism and can be used to track the evolution of diabetes, even in situations where measuring HbA1c is not advised, including diabetic nephropathy. In various fluids, 1,5-AG levels remain constant and are correlated with blood glucose levels. On the other hand, SGLT-2 inhibitors may cause artificially low readings by interfering with its measurement. Despite this, 1,5-AG helps track the development of diabetes and hyperglycemia, including microvascular problems. The non-invasive marker salivary 1,5-AG exhibits potential since it has a strong correlation with serum levels and fasting glucose, which can improve the effectiveness of diabetes screening. To confirm its usage in non-invasive diagnostic techniques for wider clinical application, more research is required (Jian et al., 2020).

e. β Cell

In all types of diabetes, the islet β cell exhibits a crucial part in the advancement of hyperglycemia. Before achieving the diagnostic criteria for diabetes, changes in the metabolism of proinsulin and insulin secretion, along with the death of β cells, are recognized as components of the hyperglycemic syndrome. Biomarkers—such as genetic markers, circulating molecules, and imaging methods—are being used more and more in research and clinical settings to predict, diagnose, and prognosticate diabetes. Functional measurements of β-cell secretory function are nevertheless frequently required despite their usefulness.

Reduced β cell count and dysfunctional secretory function in the remaining cells are the hallmarks of T2DM. This progressive process is seen when looking at function longitudinally, but because of the limitations of existing imaging methods and the ethical issues surrounding recurrent biopsies, it is less obvious when looking at β-cell bulk. Early in the pathogenic course of the disease, loss of secretory function can be seen, and diminished reactivity to secretagogues is noticeable long before glucose levels cross diagnostic limits. As the condition worsens, more and more glucose-lowering drugs are usually needed, which ultimately results in the requirement for insulin, which denotes almost complete β-cell loss.

When compared to matched patients, the majority of individuals with T2DM have reduced β-cell mass, with losses ranging from 40% to 60%. A 40% shortfall is possible even in those with impaired fasting glucose (IFG). The extent of β-cell loss, however, differs greatly throughout people, with a significant overlap in the proportion of β-cells between healthy people and those who have T2DM. The low replication rate of mature β cells is a contributing factor to this decline, which is caused by cell death and perhaps dedifferentiation. Variations in pancreatic weight, islet density, and the percentage of β cells in different pancreatic areas make quantifying β cells difficult. A precise assessment of the β-cell deficiency may not always be possible using histology on pancreatic sections alone.

Individuals with impaired glucose tolerance (IGT) or insulin-free glucose (IFG) as well as those with overt T2DM have defective β-cell secretory activity. Both pulsatile and oscillatory secretion as well as dynamic elements brought on by secretagogues are impacted by these deficiencies. Immunostaining the islet reveals that insulin is still there, but the degree of secretory dysfunction and glycemia is related to the inefficiency with which β cells convert proinsulin to mature insulin. Individual variances in the severity of the β-cell malfunction in T2DM contribute to variations in the course and consequences of the condition. Age is a significant factor: older people tend to have lower hyperglycemia, whereas younger people especially adolescents show a faster loss in β-cell activity. Compared to middle-aged adults with similar levels of body adiposity, adolescents typically have hyperresponsive β cells and are more insulin resistant.

Owing to variations in β-cell function, some patients respond well to oral treatment, while others advance quickly and need insulin therapy. Other factors that influence the variability of glucose metabolism include socioeconomic status, drugs like steroids, and sex hormones, especially the presence of estrogen. Because gene-based scores predict glucose concentrations and have a role in the development of diabetes, such as the genetic risk score (GRS) and partitioned polygenic score, they further emphasize the heterogeneity in T2DM. Variations in β-cell function can probably be attributed to heterogeneity in individual β-cell function, variations in β-cell mass between people with and without T2DM, and effects of gene variants on gene expression.

Subsequent investigations into new β-cell biomarkers, whether or not they are accompanied by functional testing, ought to more accurately identify this variability, which could unveil other distinctions and enhance their usefulness in the treatment of T2DM. Assay methodology advancements have led to the discovery of novel autoantibodies and insights into propeptide processing, which allow real-time assessment of β-cell death. The development of imaging technology has made the quantification of β cells more practical. Large datasets from longitudinal, cross-sectional, and intervention studies provide the potential for machine learning and bioinformatics techniques to improve our knowledge of diabetes progression and subtypes, bringing us one step closer to precision medicine. Notwithstanding the difficulties, there is optimism that β-cell biomarkers will soon lessen the need for functional testing in the prediction, diagnosis, and prognosis of diabetes (Kahn et al., 2021).

f. Amino Acids

Fasting branched chain and aromatic amino acids were found to be associated with obesity and serum insulin levels, while glucose loading reduced the levels of amino acids in people with insulin sensitivity but not IR people (Felig et al., 1969). Skeletal muscle's insulin-mediated inhibition of proteolysis is most likely to blame for this. Recent research has indicated a connection between amino acids and obesity, insulin resistance, and prediabetes. An elevated risk of diabetes has been closely linked to the aromatic amino acids phenylalanine and glycine, as well as the branched-chain amino acids (BCAAs), isoleucine, leucine, valine, and tyrosine. Moreover, insulin-resistant conditions are linked to increased levels of glutamine, methionine, cysteine, and 2-aminoadipic acid. On the other hand, those with prediabetes had lower glycine levels. Further support for this came from a comprehensive

systematic review and meta-analysis, which demonstrated favorable correlations with aromatic amino acids and BCAAs and inverse links with the risk of T2DM and glutamine and glycine. Variations in blood amino acid levels may be a key sign of both T2DM and IR (Dorcely et al., 2017).

g. C-reactive protein (CRP) and Interleukin-6 (IL-6)

Two important markers of inflammation that have been extensively studied in heart disease and diabetes are interleukin-6 (IL-6) and CRP (c-reactive protein). IL-6-dependent hepatic activity is the main factor that produces CRP during acute inflammation; increased CRP and IL-6 levels are common in individuals with T2DM mellitus (T2DM) and IR. A higher risk of T2DM has been associated with higher baseline levels of CRP and IL-6, as demonstrated by studies such as the Insulin Resistance Atherosclerosis Study (IRAS) and the Women's Health Study. In the Women's Health Study, higher quartiles of IL-6 and CRP were linked to an increased risk of incident T2DM even after adjusting for factors including BMI. Similar trends were observed in the IRAS, where individuals with prediabetes and insulin resistance exhibited greater CRP levels than their insulin-sensitive counterparts; irrespective of their glycemic state. Numerous other investigations, including the Gutenberg Health Study, which underlined the importance of early immunological activation in the establishment of diabetes, have also validated the association between CRP and prediabetes. T2DM susceptibility and CRP levels are also influenced by genetic differences in the innate immune system.

The relationship between T2DM risk and IL-6 and CRP levels has been further supported by meta-analyses. Although IL-6 appears to be more strongly connected with T2DM than T2DM, CRP is raised in obesity and insulin resistance, indicating a complicated interplay of pathways of inflammation in the pathophysiology of diabetes (Dorcely et al., 2017).

h. Lipoprotein (a) (LP(a))

The liver synthesizes lipoprotein (a), and high levels of LP(a) constitute a stand-alone risk factor for cardiovascular diseases [9]. There is evidence of an inverse correlation between the prevalence of T2DM and prediabetes and serum Lp (a); large population studies like the Women's Health Study (WHS) and Copenhagen City Heart Study (CCHS) have been generating a lot of interest due to an inverse relationship between serum Lp(a) concentrations and the risk of T2D. Furthermore, epidemiologic evidence linking blood Lp(a) levels to T2D, particularly in Asian populations, however, the exact mechanism behind this link is unclear Lp (a) levels may be decreased in part by insulin (Ding et al., 2015).

i. Triglycerides and High-Density Lipoprotein

In prediabetes, decreased insulin production and β-cell dysfunction have been linked to elevated blood triglyceride (Tg) levels. Mechanistically, hypertriglyceridemia inhibits the glucose-induced release of insulin via the glucose-fatty acid cycle and encourages the death of cells by increasing nitric oxide and ceramide synthesis. Elevated levels of Tg can lead to lipotoxicity by building up inside pancreatic cells (Dorcely et al., 2017).

High-density lipoprotein (HDL) and Tg-rich lipoproteins exchange lipids through the medium of cholesterol ester transfer protein. This exchange is accelerated in insulin-resistant conditions by elevated Tg levels. Subsequently, hepatic lipase hydrolyzes the Tg in HDL cholesterol (HDL-C), producing smaller HDL-C particles. Small HDL3 particles are the target of cholesterol efflux, which is mediated by ATP-binding cassette transporter A (ABCA1). Compared to HDL-C levels, subjects with prediabetes had noticeably higher quantities of tiny HDL3 particles [19] HDL-C and Tg have a negative correlation with the proportion of tiny HDL3 particles, while the opposite is true for HDL-C. Through its association with ABCA1, HDL-C, in contrast to Tg, stimulates insulin secretion. Progression from prediabetes to diabetes can also be caused by low HDL-C concentrations. Regarding β-cell dysfunction, it is uncertain whether HDL-C levels are related [8]. Degrading phospholipids that have been oxidatively fragmented, lipoprotein-associated phospholipase A2 (LpPLA2) is an enzyme that may be involved in atherogenesis. When comparing participants with normoglycemia to those with IFG, HDL-associated LpPLA2 (HDL LpPLA2) activity is considerably lower. While LpPLA associated with low-density lipoprotein (LDL) may have two pro-inflammatory effects, LpPLA2 connected with HDL may have an atheroprotective function. In individuals with IFG, there was a decrease in the anti-atherogenic HDL's activity and an increase in tiny HDL3 particles. Consequently, prediabetes may be influenced by HDL-C subtypes LpPLA2 (Carstensen et al., 2010).

j. Ceramide

Ceramides are positively correlated with T2DM and prediabetes in addition to Tg. Lipid compounds called ceramides mediate IR. Ceramides accumulate in insulin-resistant tissues, limit the action of insulin by lowering Akt **(**Protein Kinase B**)** phosphorylation and activation, and cause inflammation through the TNF-α – nuclear factor-κB (NF-Kβ) axis, according to studies. Ceramides and coronary artery disease are also correlated. To fully comprehend the connections between lipid metabolism, prediabetes, and diabetes, more research is required (Dorcely et al., 2017).

Using oxidative mechanisms, high blood sugar levels immediately increase the amounts of cytokines, including plasma IL-6, TNF-α, and IL-18. In a prospective case-cohort analysis, subjects in the

highest quartile of IL-18 had a 70% greater likelihood of developing T2DM than those in the lowest quartile. In a study, when prediabetes gave way to diabetes, IL-18 also increased (Dorcely et al., 2017).

k. IL-1 receptor antagonist

FFAs and hyperglycemia during overindulgence may trigger the IL-1 pathway, which might result in an inflammatory condition. In prediabetes and diabetes, there is an increase in the anti-inflammatory marker IL-1 receptor antagonist (IL1RA), which is produced by adipocytes; this rise may represent a reactive response to inflammation (Khan et al., 2019). Higher levels of IL-1RA were observed in prediabetes along with lower insulin sensitivity, temporarily elevated β-cell function, and 2-hour glucose levels—all of which occurred years before the development of T2DM—in a study involving 355 individuals who had incident T2DM (Christian et al., 2009). Thirteen years before T2DM developed, there were greater levels of IL-1RA. Surprisingly, IL-1RA increased significantly six years before diagnosis, even after considering weight (Christian et al., 2009).

CONCLUSION

Regardless of the scientific advancements in the healthcare industry, T2DM remains a complicated condition that requires further insight and advancements to effectively diagnose and treat patients. The currently implicated diagnostic biomarkers are useful however, they possess a range of disadvantages as well, especially those used in the early detection of the disease. One of the most challenging aspects of the disease is the lack of early diagnosis which causes the development of severe complications. This indicates the need to develop novel, reliable, precise, and disadvantage-free biomarkers. There is a wide range of newly emerging biomarkers such as miRNAs, inflammatory mediators, and cellular metabolites claiming to be strong candidates however further research is required to validate their clinical implications The development of novel, reliable, precise, and disadvantage-free biomarkers is essential.

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