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Emerging Diabetic Novel Biomarkers of the 21st Century

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Abstract

Diabetes is a growing epidemic with estimated prevalence of infected to reach ~592 million by the year 2035. An effective way to approach is to detect the disease at a very early stage to reduce the complications and improve lifestyle management. Although several traditional biomarkers including glucated hemoglobin, glucated albumin, fructosamine, and 1,5-anhydroglucitol have helped in ease of diagnosis, there is lack of sensitivity and specificity and are inaccurate in certain clinical settings. Thus, search for new and effective biomarkers is a continuous process with an aim of accurate and timely diagnosis. Several novel biomarkers have surged in the present century that are helpful in timely detection of the disease condition. Although it is accepted that a single biomarker will have its inherent limitations, combining several markers will help to identify individuals at high risk of developing prediabetes and eventually its progression to frank diabetes. This review describes the novel biomarkers of the 21st century, both in type 1 and type 2 diabetes mellitus, and their present potential for assessing risk stratification due to insulin resistance that will pave the way for improved clinical outcome.

Keywords
- 21st century
- biomarkers
- review
- type 1 diabetes mellitus
- type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a complex metabolic and multifunctional syndrome that is characterized by persistent hyperglycemia. The prevalence of DM has increased over the past two decades and in coming years, it will be a major health problem for the world.[1] According to the International Diabetes Federation report, prevalence of diabetes is spiraling globally with an estimated 425 million adults having diabetes in 2017 and will be increased to ~592 million by the year 2035.[2] Developing countries like India will have ~109 million of affected people, making diabetes no more a developed world disease.

DM has a multifaceted pathogenesis that occurs either due to impaired insulin secretion or due to development of insulin resistance (IR) at target tissues or because of insulin deficiency due to autoimmune destruction of pancreatic β-cells.[3]

DM is classified into different types based on the pathogenic mechanisms as type 1, type 2, gestational diabetes, and other types in which specific, genetic defects, metabolic, and mitochondrial abnormalities and some conditions that impair glucose tolerance are included.[4]

Diabetes, if untreated, may lead to several serious chronic complications involving microvascular complications like nephropathy or retinopathy and macrovascular complications like cardiovascular disease (CVD) and stroke. The most common cause of mortality in diabetes is, however, macrovascular complications.[5]

Given the burden of diabetes and its complications, much attention has been given to prevention, beginning with

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identifying at-risk individuals prior to diagnosis that led to the designation of "prediabetes," an intermediate form of dysglycemia on a spectrum ranging from normal to overt diabetes.[6] According to Centers for Disease Control, one out of three adults had prediabetes and agonizingly, 90% were unaware of their diagnosis.[7]

Awareness and knowledge regarding the diabetic condition, its management, complications, and risk factors are crucial steps for better quality of life and its control.[8]

However, the clinical manifestations especially in type 2 diabetes mellitus (T2DM) are delayed by years, thereby restricting its timely diagnosis. It has been demonstrated that there is a significant relative risk reduction of CVDs and all-cause mortality in individuals undergoing regular medical examination emphasizing the importance of early diagnosis of the disease.[9]

The efficacy of diabetic management to delay progression of DM would be improved if they could be implemented during the initial phases of the disease and number of studies also suggest that progression of diabetes can be postponed or prevented with earlier initiation of current treatment protocols.[10,11] Thus, the prediction and early identification of high-risk individuals before the onset of prediabetes stage, that is, when the β-cells are relatively intact, along with timely identification of diabetic complications, are of paramount importance, for effective intervention. Timely management though is helpful in preventing progression to overt disease but, however, remains a challenging scenario.

Considering the need of present situation, prompt diagnosis and innovation of an effective and safe treatment option are required to achieve this goal, identifying new biomarkers for predicting individuals at high risk of diabetes and its complications have, therefore, become a priority for targeting preventive measures efficiently.[12,13]

Thus, this review is intended to discuss the role of novel biomarkers of 21st century in patients of DM as predictor of diabetic risks or to detect earlier diabetic complications so as to aid in better understanding of disease course propose safe and effective therapeutic interventions. Various biomarkers are shown in ►**Fig. 1**.

Traditional Biomarkers

Hemoglobin A1c

Chronic glycemia is better estimated by hemoglobin A1c (HbA1c) than glucose levels at a single time point and currently is the most commonly used biomarker for diagnosing prediabetes and diabetes. American Diabetes Association criteria for diabetes are HbA1c ≥6.5% (48 mmol/mol) and 5.7 to 6.4% (39–46 mmol/mol) for prediabetes.[14] In the Norfolk prospective study, higher HbA1c levels were associated with increased CVD and all-cause mortality.[15] HbA1c may be a better predictor of microvascular complications than fasting plasma glucose (FPG).[16] Other advantages of HbA1c over FPG and oral glucose tolerance test (OGTT) are its greater convenience as fasting is not required, greater preanalytical stability, and less day-to-day perturbation during periods of stress and illness.[17] Thus, HbA1c is particularly useful for lifestyle modification counselling as it reflects chronic exposure to glucose but, however, there is conflicting evidence regarding its usefulness in diagnosing diabetes in comparison to OGTT and FPG as it provides moderate sensitivity.[17,18] The National Health and Nutrition Examination Survey and Screening for Impaired Glucose Tolerance studies showed HbA1c levels <5.7% (39 mmol/mol) correlate only 60 to 70% of subjects having normal glucose tolerance.[19-21] Moreover, HbA1c threshold for prediabetes does not take ethnicity, body mass index (BMI), and age, all of which may significantly alter HbA1c levels under consideration.[22-25] OGTT more strongly correlates with IR and insulin secretion than HbA1c.[26] HbA1c is also not always a reliable measurement of average circulating glucose levels as changes in the production rate or circulating life span of red blood cells will affect HbA1c levels; for example, it may be falsely elevated in iron deficiency anemia, folate and vitamin B12 deficiency, severe hypertriglyceridemia conditions where reduced production leads to a greater percent of older cells, whereas false low values may been seen in conditions of rapid turnover of red blood cells like in HbA1c occurs in splenomegaly and end-stage renal disease.[27] Hemoglobin variants, such as HbS, HbC, HbD, and HbE, may also result in overestimation or underestimation

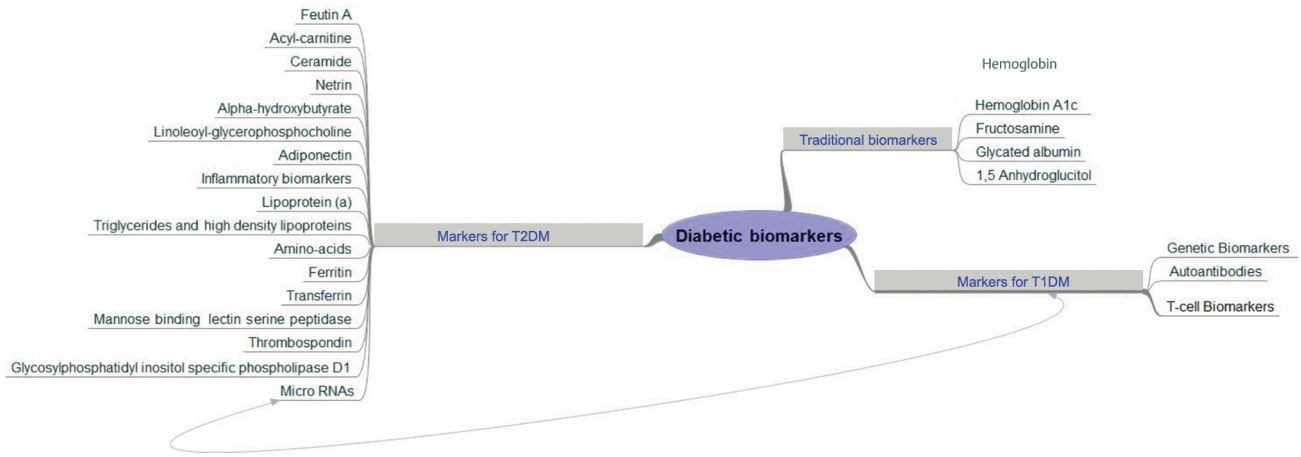

Fig. 1 Biomarkers in diabetes mellitus.

of HbA1c.[28] So, HbA1c alone cannot be considered adequate for diagnosing prediabetes, and more accurate diagnosis may require confirmation with other biomarkers.[29]

Fructosamine

Fructosamine (FA) is a ketoamine formed by glycosylation of fructose to total serum protein, mostly albumin.[30] It reflects average blood glucose concentrations over the previous 1 to 4 weeks, and thus can be a useful clinical marker of short-term glycemic fluctuation and glucose control.[31] Currently, it is used as an alternate glycemic marker for diabetes screening. FA could be a valuable complementary marker in clinical conditions, where HbA1c may be inaccurate or it may also play a role in identifying fluctuating glucose levels in DM patients with stable HbA1c along with other measures.[32,33] FA measurement is cost-effective and convenient as it does not require fasting[34,35] and is advantageous in conditions that affect hemoglobin levels.[36] However, limitations include, higher variations within the subjects[37] and falsely low values are seen physiologically in young children who have lower serum protein concentration than adults and pathologically in conditions with rapid albumin turnover as in nephrotic syndrome, severe liver disease, or protein-losing enteropathy.[38] Though it is useful to diagnose prediabetes, some studies also doubt its usefulness for prediabetes screening.[31,39-41] Thus, FA can serve as a useful alternate biomarker under specific conditions.

Glycated Albumin

Glycated albumin (GA) measures the ratio of GA to total albumin and thus it is better than FA in clinical conditions that result in protein loss such as nephrotic syndrome, liver, and thyroid disease.[42] It is also considered to be a better index of glycemic control than HbA1c in patients with renal failure, hemolytic anemia, and those receiving blood transfusions.[43,44] Due to shorter half-life of albumin, GA reflects a shorter glycemic control, of 2 to 3 weeks as compared with HbA1c.[45] The combination of GA with HbA1c is a more sensitive predictor of prediabetes than HbA1c alone.[46] The speed of glycation of GA is 10 times faster than HbA1c; thus, its value better reflects variations in blood glucose and postprandial hyperglycemia in combination with HbA1c.[47] GA estimation is of limited use in conditions of abnormal albumin metabolism.[48] Other disadvantage is that sometimes GA may be artificially low in individuals having increased BMI, body fat mass, and high visceral fat.[49]

1,5-Anhydroglucitol (1,5-AG)

It is the naturally occurring, 1-deoxy form of glucose whose plasma levels are inversely correlated with plasma glucose levels. Renal proximal tubules have relatively greater affinity for glucose than 1,5-AG. Thus, plasma concentrations of 1,5-AG decrease due to its increased urinary concentration, as studied in healthy control, prediabetes, and diabetes groups.[50] It has been suggested as a prediabetes marker and reflects glucose levels within the preceding 10 to 14 days. AG has the advantage of being stable, reproducible, and less costly when compared with other glycemic diagnostic tests and may be useful for identifying postprandial glycemic excursions and individuals at risk of microvascular and macrovascular complications in diabetes.[51] However, plasma 1,5-AG levels can change based on diet, sex,[52,53] and race and also fluctuating levels have been seen in patients receiving SGLT 2 inhibitors or those on renal replacement therapy.[54,55] Also, some studies disagree with the use of 1,5-AG as a prediabetes screening tool. On the contrary, a few studies disagree with the use of 1,5-AG as a prediabetes screening tool.[53,54]

Novel Biomarkers for Type 2 Diabetes Mellitus

Fetuin A

Fetuin-A, also called alpha 2-Heremans Schmid glycoprotein, is a physiological inhibitor of insulin receptor tyrosine kinase and thus associated with IR, metabolic syndrome and an increased risk for T2DM.[56,57] Pal et al showed that fetuin-A binds to toll-like receptor 4 (TLR4)-inflammatory signaling pathway, which results in production of inflammatory cytokines, and, thus, promotes lipid-induced IR through this interaction.[58] Fetuin-A knockout mice have also demonstrated increased basal and insulin-stimulated phosphorylation of insulin receptor, increased glucose clearance, and improved insulin sensitivity.[59,60] As measured by the index of homeostasis model (HOMA-IR), fetuin-A is found to be an independent determinant in the development of IR.[61-63] It is well known that IR has been identified as the major pathophysiologic determinant of T2DM.[64,65] In cross-sectional studies, genetic analyses revealed that SNPs in the fetuin-A gene (located at a susceptibility locus for T2DM) are linked to T2DM.[66,67] Both the Health, Aging and Body Composition Study (Health ABC) and European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study indicate that participants with high fetuin-A levels have an increased risk of incident diabetes.[68] However, there have been conflicting reports regarding association between fetuin-A concentration and CVD.[69-72] Thus, taken together, fetuin-A acts as an endogenous ligand for TLR4 through which lipids induce IR and may serve as a novel therapeutic target for IR.

Acyl-Carnitine

L-carnitine, a small water-soluble molecule, plays an essential role in intermediary metabolism, by transporting long-chain fatty acids from the cytosol into the mitochondria, where their degradation takes place via β-oxidation.[73] Beyond this, other crucial functions in the body are modification of acyl-(conenzyme A) CoA/CoA ratio, energy storage in the form of acetyl carnitine, anti-inflammatory and antioxidant properties. It also improves insulin sensitivity, dyslipidemia, and membrane stability.[74] Recently, serum levels of acylcarnitines have been shown to be elevated in prediabetes.[75,76] Lipid oversupply results in accumulation of incompletely metabolized fatty acids in the mitochondria causing "mitochondrial stress," leading to IR.[77] According to another theory, the long-chain acyl-CoAs are precursors of ceramide that is already being explored as affector of insulin resistance.[78] Recent studies suggest an alternative mechanism

in which fatty acid oxidation (FAO) rate outpaces that of the tricarboxylic acid cycle, resulting in the accumulation of intermediary metabolites, such as acylcarnitines, which may affect insulin sensitivity.[74] A recent Canadian study reveals that an elevation in circulating medium chain acylcarnitines is associated with gestational DM and early stages of T2D onset and that this elevation directly impairs β-cell function.[79] Another study also showed acylcarnitine profile, mainly including short- and long-chain acylcarnitines, was significantly associated with higher T2D risk in participants at high cardiovascular risk.[80]

Ceramides

Ceramides, a type of sphingolipid with long chain fatty acid of varying length, constitute major component of the biological membrane. Ceramides are available in the human system by de novo pathway, salvage pathway, and by sphingomyelin hydrolysis. Their accumulation may increase in tissues due to excessive supply of fatty acids, mostly as a result of sphingolipid salvage pathway activity.[81] Apart from its structural role in biological membranes and signal transduction, ceramides can also antagonize insulin signaling by inhibiting transmission of signals through phosphatidylinositol-3 kinase and blocking activation of the anabolic enzyme Akt/PKB. They also stimulate caspase, protein kinase C, serine/threonine protein phosphatase, and cathepsin D activity.[82] Thus, ceramides interfere with glucose uptake, impair storage of nutrients such as glycogen or triglyceride (Tg), activate proinflammatory cytokines, disrupt lipid metabolism, and even enhance cell death (proapoptotic). Plasma ceramides have been found to be increased in obesity and insulin resistance (IR).[83] Insulin also elicits an anabolic effect on sphingolipid metabolism, resulting in increased ceramide accumulation in skeletal muscles.[84] This shows a deleterious consequence of the hyperinsulinemia that accompanies IR leading to a vicious cycle. A study suggests that ceramide stearic to palmitic acid ratio Cer (d18:1/18:0)/Cer(d18:1/16:0) is an independent predictive biomarker for new onset diabetes mellitus even years before diagnosis; thus it may be modulated by lifestyle intervention.[85] Since ceramides are detectable in easily accessible body fluids, they have recently been proposed as promising biomarker candidates in several diseases such as cancer, multiple sclerosis, Alzheimer's disease, and coronary artery disease and T2DM.[86]

Netrin

Netrin is a family of extracellular, laminin-related proteins,[87] comprising of netrin-1, netrin-3, and netrin-4, and two glycosylphosphatidylinositol (GPI) anchored membrane peptides (netrin G1 and G2).[88] They are expressed in the central nervous system and also in nonneural tissues such as vascular endothelial cells, pancreas, liver, spleen, lung, intestine, and kidney.[89] Keeping in view its pathogenic role, netrin is emerging as a novel diagnostic and prognostic biomarker for variety of life-threatening diseases like cancers, cardiovascular diseases, acute kidney injury, and subarachnoid hemorrhage.[90] As netrin-1 is involved in pancreatic morphogenesis, islet-cell migration, and rejuvenation, it was likely that they have a role in diabetes as well. A recent clinical study found a significant increase of serum Netrin-1 level in subjects with impaired fasting glucose (IFG) or T2DM compared with the control group suggesting that Netrin-1 may be used as a biomarker for their early detection.[91] On the contrary, another study found that the level of Netrin-1 in diabetic patients was significantly reduced than that of healthy controls.[92] However, netrins, having role in angiogenesis and inflammatory process, show a promising role as marker for early detection and prognosis of diabetic microvascular complications like retinopathy and nephropathy.[90] Netrin-1 regulates corneal epithelial wound healing, inflammation response, and nerve fiber regeneration in diabetic mice, indicating the potential application for the therapy of diabetic keratopathy.[91] They have also shown beneficial efficacy in the treatment of diabetic nephropathy.[92]

α-Hydroxybutyrate and Linoleoylglycerophosphocholine

Recently increased levels of plasma α-hydroxybutyrate (α-HB), an organic acid, and decreased levels of plasma linoleoylglycerophosphocholine (L-GPC), a lipid, have been described as joint markers of peripheral IR and glucose intolerance, as measured by the euglycemic hyperinsulinemic clamp technique—in selected subjects in the Relationship between Insulin Sensitivity and Cardiovascular Disease study, a cohort of well-phenotyped nondiabetic individuals.[93] α-Hydroxybutyrate (α-HB) is a catabolic by-product of threonine, methionine, and glutathione anabolism (cysteine formation) in hepatic tissue.[93] Increased oxidative stress and lipid oxidation lead to chronic shifts in glutathione synthesis resulting in elevated α-HB levels in individuals of IR.[94,95] Thus, it is suggested that α-HB, a proximate product of disordered metabolism, might serve as both a predictive biomarker and prodromal sign of incipient T2DM.[96]

L-GPC is formed by hepatic phospholipase A2 and circulatory lecithincholesterol acyltransferase. Choline-containing phospholipids and sphingomyelins have been associated with increased risk of T2DM.[97,98] L-GPC is an independent correlate of insulin sensitivity and a putative lipid-signaling molecule.[93]

Both the above biomarkers of insulin sensitivity are independently associated with glucose intolerance.[93] However, L-GPC is a negative predictor of T2DM progression in contrast to αHB, a positive predictor.[93,99] But both the biomarkers can be used in predictive models to identify subjects with impaired glucose tolerance that is a high-risk state for the development of T2DM, without performing an OGTT.[97]

Adiponectin

Adiponectin is a protein exclusively expressed in differentiated adipocytes, cardiac tissue, bone, mammary and salivary glands.[100] It has insulin sensitizing, anti-inflammatory, and anti-atherogenic functions and it is shown to be independent predictor of diabetes.[101] It also has antiangiogenic and possibly anticancer properties.[102] Adiponectin may be an important modulator of insulin sensitivity through increased oxidation of fatty acid, glucose uptake and utilization in skeletal muscle and adipose tissue.[103] High level of adiponectin

protects glucose metabolism impairment in obese patients and decreases the risk of T2DM development.[104] Level of adiponectin is found to be lower in T2DM and obese subjects.[105] In a cohort-based study of subjects with impaired glucose tolerance, low adiponectin level was found to be a strong independent predictor of diabetes.[106] In offspring of diabetic parents, the baseline adiponectin levels are inversely related to the risk of prediabetes and it is independent of sex or ethnicity.[107] Adiponectin administration has found lower levels of plasma glucose and increased insulin sensitivity.[108] On the other hand, treatment of patients with insulin increased the level of circulating adiponectin and improved insulin sensitivity.[104]

Inflammatory Biomarkers

Inflammatory biomarkers are involved in the activation of innate and adaptive immune systems that are recognized as key mediators in diabetes development. Specifically, acute phase proteins (C-reactive protein [CRP] or high-sensitivity [hs-CRP] and fibrinogen) and pro-inflammatory cytokines (interleukin-6 [IL-6] and tumor necrosis factor-α) are associated with increased risk of T2D.[109] Elevated levels of IL-6 and CRP are useful in identifying individuals at higher risk of developing T2DM.[110] Genetic variants in the innate immune system and inflammatory cascade also affect CRP and predisposition to T2DM.[111,112] A meta-analysis of genome-wide association study on hs-CRP showed shared genes and pathways that are associated with IR and T2D.[113,114] Tissue plasminogen activator-1 change is an independent predictor of incidence of diabetes.[115] IL-18 levels also increased with progression from prediabetes to diabetes in the Gutenberg study.[116] The IL-1 receptor antagonist (IL-1RA), produced by adipocytes, is an anti-inflammatory marker elevated in prediabetes and diabetes, possibly as a reactive response to inflammation. The Whitehall Study, has also shown an increase in IL-1RA in prediabetes in parallel with decreasing insulin sensitivity, increasing β-cell function, and 2-hour glucose levels, all of which occurred altogether years before the development of T2DM.[116] Patients with T2DM frequently have dysfunctional coagulation profile, especially increased fibrinogen levels that are independently associated with HbA1c values.[117] Fibrinogen levels were also seen to be associated with diabetic nephropathy, but not with other microvascular complications like retinopathy. With respect to macrovascular disease, the association between high plasma fibrinogen and peripheral vascular disease is seen in T1DM.[118]

Lipoprotein(a)

Lipoprotein (a) [Lp (a)] is a plasma lipoprotein that consists of LDL-like particle, consisting of apo (a) that is covalently attached to one molecule of apoB100 via a disulfide bond and is synthesized in liver.[119] Lp(a) levels are primarily under genetic control, with genetic variants and the number of Kringle IV-2 repeats in the LPA gene accounting for much of the variability in Lp (a) concentrations.[120] Elevated levels of LP(a) are proved to be independent risk factor for the development of CVD.[121] Increase in Lp (a) levels may promote atherosclerosis via Lp (a)-derived cholesterol entrapment in the intima, via inflammatory cell recruitment, and/or via the binding of proinflammatory-oxidized phospholipids.[122] Inverse relationship between serum Lp (a) and T2DM, prediabetes, IR, and with hyperinsulinemia has also been noted.[123] Though the mechanisms through which Lp(a) might be inversely associated with diabetes risk is not yet clear, there is some evidence that Lp(a) is a marker of, or might be involved in, the development of IR.

Amino Acids

Amino acids have emerged as novel biomarkers in the identification of people at risk of T2D before overt symptoms. With advancement in technologies involving metabolic profiling, circulating amino acids may illustrate new pathways in diabetes pathophysiology like bidirectional modulation of insulin action due to crosstalk between hormonal and nutritional signals and may suggest novel mechanism by amino acids.[124,125] More recent studies have demonstrated a correlation between amino acids and prediabetes, IR, and obesity.[93] It is shown that serum branched chain amino acids and aromatic amino acids are significantly and positively associated with T2D.[126] In addition, glutamine, methionine, cysteine, and 2aminoadipic acid are increased in insulin resistant states.[127-129] By contrast, glycine levels are decreased in individuals with prediabetes.[130-132] Thus, changes in these circulating amino acid levels may prove to be significant predictive markers for T2D.

Triglycerides and High-Density Lipoprotein

Elevated serum Tg and subclasses of high-density lipoprotein cholesterol (HDLC) maybe involved in the pathogenesis of diabetes. Hypertriglyceridemia has been associated with β-cell dysfunction and reduced insulin secretion in prediabetes.[121] Tg levels can cause lipotoxicity within pancreatic β-cells and may lead to β-cell apoptosis by stimulating the production of ceramide and nitric oxide.[121,133] Levels of small HDL-3 particles have been found to be elevated in prediabetic subjects as compared with HDLC levels. The proportion of small HDL-3 particles is positively associated with Tg and negatively associated with HDLC.[134] However, unlike Tg, low HDLC concentrations may also lead to progression to diabetes from prediabetes, but its association with β-cell dysfunction is unclear.[121]

Ferritin and Transferrin

Both ferritin and transferrin levels are associated with hyperinsulinemia and hyperglycemia.[135] A recent study in healthy women showed that higher iron stores as reflected by ferritin concentrations and the ratio of transferrin receptors to ferritin were associated with an increased risk of T2DM, independently of known diabetes risk factors.[136] Iron contributes to IR through the production of highly active radical formation, damage to DNA and cell membrane integrity, β-cell oxidative stress resulting in decreased insulin secretory capacity, and interference with glucose uptake in skeletal muscles and adipocytes. Dietary iron restriction prevents the development of diabetes and loss of β-cell function.[137] Not

only iron stores but also iron transport is involved in the development of the metabolic syndrome and very likely of IR.[138] Transferrin has been shown to be a major determinant of lipolytic activity in adipocytes by a pro-oxidative mechanism.[139] and adipose tissue lipolysis has been recognized as a major determinant of IR.[140] Higher levels of transferrin are usually negatively correlated with ferritin.[135]

Mannose-Binding Lectin Serine Peptidase

Mannan-binding lectin serine protease 1 also known as mannose-associated serine protease 1 (MASP-1) is involved in the lectin pathway of the complement system and is responsible for cleaving C4 and C2 into fragments to form a C3-convertase.[141] MASP-1 is also able to cleave fibrinogen and factor XIII and may be involved in coagulation.[142] Thus, elevated levels of these proteins may play a role in the enhanced thrombotic environment and consequent vascular complications in diabetes.[143] It has been observed that the onset of prediabetes and IR occurred earlier in those with increased MASP-1 plasma levels. Elevated FPG and 2-hour glucose levels also have positive association with higher levels of MASP-1.[144] Thus, these proteins have a potential role as a biomarker to detect early macrovascular complications in diabetes.

Thrombospondin

Thrombospondins (TSP 1–5) are a group of multifunctional secretory glycoproteins. TSP-1, a potent antiangiogenic and proatherogenic protein, has been implicated in the development of several vascular diabetic complications.[145] TSP-4 is an extracellular matrix protein of the vessel wall.[146] Endogenous TSP-1 protects the myocardium from infarction-induced and pressure overload-induced cardiac remodeling.[147] TSP-1 is also an adipokine that is highly expressed in obese, insulin-resistant subjects; and it is highly correlated with adipose inflammation.[148] In the diabetic setting, decrease in TSP increases matrix metalloproteinase (MMP-2 and MMP-9) activities and suppresses fibroblast function thus inhibiting collagen synthesis.[149] They could be a novel marker for atherosclerotic burden, especially in the major subgroup of patients with concomitant diabetes.[150] TSP-1 plays an important role in the development of complications in patients with diabetic nephropathy and its serum level is related to renal injury and vascular disease.[151]

Glycosylphosphatidylinositol Specific Phospholipase D1

Glycosylphosphatidylinositol-specific phospholipase D (GPLD1, also called GPI-PLD) is a 110- to 120-kDa N-glycosylated amphiphilic protein abundant in mammalian serum, where it associates with HDL.[152] The association with HDL raises the possibility that GPI-PLD may be involved in lipid/lipoprotein metabolism. GPLD1 may be regulated by several different hormones or metabolites involving circulating insulin levels, hyperglycemia, oxidative stress, and inflammation.[152,153] In addition, GPI-PLD appears to colocalize with insulin in the secretory granule,[154] raising the possibility that diabetes may be associated with changes in islet production of GPI-PLD. Recent evidence suggests that GPLD1 levels are increased in circulation following the onset of diabetes induction and IR both in rats and humans, respectively, suggesting its association with diabetes-induced impairments.[155,156] GPLD1 may play an important role in inflammation and in the pathogenesis of diabetes. Recent study suggests the potential role of GPLD1 as a candidate plasma protein that can be used effectively to distinguish between early stage latent autoimmune diabetes in adults and T2DM.[157]

Micro-RNAs

Micro-RNAs (miRNAs) are small (~22 nucleotides) noncoding RNA sequences that inhibit gene expression of specific mRNA targets.[158] It has been reported that distinct miRNA expression profiles regulate various physiological and pathological conditions.[159] Recently, miRs have been studied in prediabetes and found to be strongly correlated.[160,161] Moreover, they could be differentially expressed in various types of DM, suggesting their potential to serve as diabetic indicators. It has been reported that miR-20b, miR-21, miR-24, miR-15a, miR-126, miR-191, miR-197, miR-223, miR-320, and miR-486 were downregulated, while miR-28–3p was upregulated in plasma of T2DM patients in comparison with non-DM individuals.[162] More recent studies also detected significantly increased levels of circulating miR-146a[163] and miR-126[164] in newly diagnosed T2DM patients compared with healthy controls. Other miRNAs significantly elevated in T2DM and found to negatively regulate insulin expression, production, or secretion include miR9, miR29a, miR30d, miR34a, miR124a2, miR146a, and miR375.[165]

In patients with recent-onset T1DM, the most consistently upregulated miRNAs were miR-152, miR-181a, and miR-27b, while miR-375 was consistently downregulated in independent cohorts.[166] miR-25, miR-24–3p, let-7 g-5p, and miR-93–5p were either upregulated or downregulated in various recent-onset T1DM cohorts.[167]

Recent studies suggest brain as a key player in glucose regulation and the pathogenesis of metabolic disorders such as T2D.[168] There is evidence suggesting that increased activation of hypothalamus-pituitary-adrenal axis and sympathetic nervous system, and consequent elevation of stress hormones, may be important for the etiology and development of T2D. The association between these neuroendocrine stress response-related circulating miRNAs and T2D has been explored by Liang et al, which may act as potential biomarkers for prediabetes and IR in adults.[169]

Thus, our understanding of the importance of miRNAs in the pathogenesis of diabetes has grown substantially; there is increasing evidence for a potential role of miRNAs as clinical biomarkers of the T1DM, pre-DM, T2DM, and gestational DM. Moreover, discovery of new set of miRNA biomarkers might help to guide diagnostic and therapeutic decisions.

Possible Biomarkers for Type 1 Diabetes Mellitus

Immune biomarkers of T1DM are diverse. Though some of them like autoantibodies are well established, they are not discriminative enough to deal with the heterogeneity

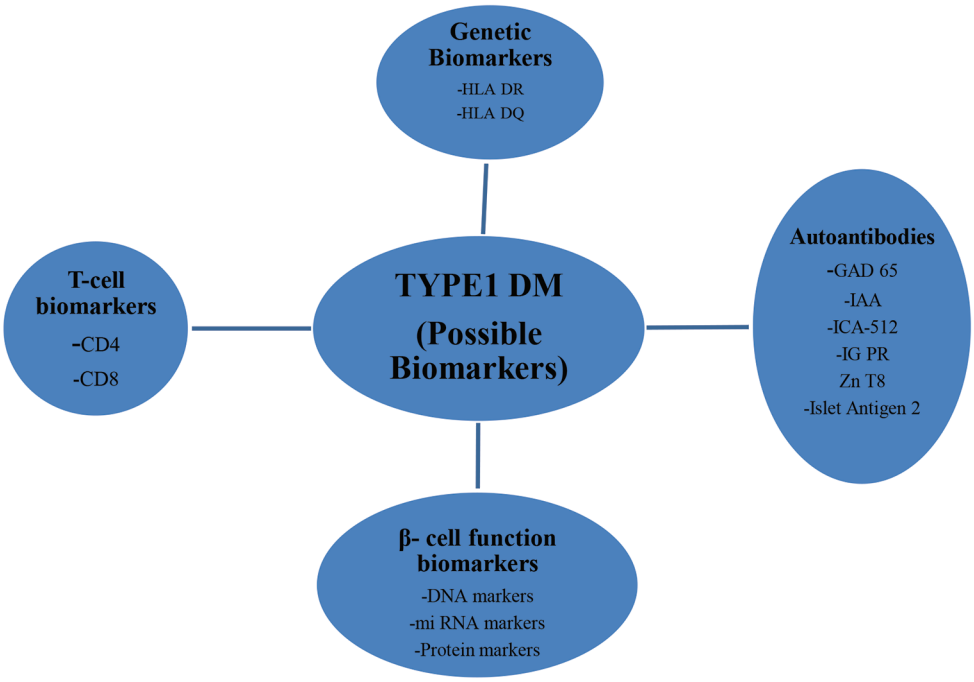

Fig. 2 Possible biomarkers in T1DM. GAD-65, glutamic acid decarboxylase-65; IAA, insulin-associated autoantibodies; ICA-512, internal carotid artery-512; IGPR, islet specific glucose-6-phosphatase catalytic subunit related protein; T1DM, type-1 diabetes mellitus; ZnT8, zinc transporter 8.

that is inherent in T1DM. As an alternative, various possible biomarkers have recently been identified that not only provide a better understanding and progression of T1DM but also yield therapeutic efficacy of immune interventions. ►**Fig. 2** highlights various biomarkers in T1DM.

Genetic Biomarkers

Genetic markers may be helpful in assessing the predisposition for T1DM, where identification of over 50 loci contributing to T1DM risk has been identified in studies of large cohorts.[170] For routine screening purpose, HLA typing is done. Though HLA typing alone is useful for the enrichment of future cases of T1DM, it is insufficiently sensitive and specific to be a biomarker for future prevention strategies. However, HLA-DR (DR3/4) and HLA-DQ (DQ8) genotypes are useful in predicting the risk of developing β-cell autoimmunity. The highest risk of HLADR/-DQ genotypes is present in around 30 to 40% of individuals with T1DM and around 2 to 3% of the background population.[171]

Autoantibodies

Autoantibodies are now widely accepted as the hallmark of T1DM. Autoantibodies against β-cell proteins and peptides for diagnosis of T1DM, are routinely used.[172] The most commonly measured are autoantibodies to glutamic acid decarboxylase, insulin-associated autoantibodies, insulinoma-associated protein 2 (IA-2, previously known as ICA-512), islet specific glucose-6-phosphatase catalytic subunit related protein, a tyrosine phosphatase like protein (islet antigen-2 [IA-2] and the most recently described zinc transporter 8.[173,174] However, the development of autoantibodies against multiple β-cell antigens is recognized as a critical step in the disease pathogenesis and is associated with a significantly higher T1DM risk than the presence of just a single autoantibody.[175,176] Autoantibody characteristics that allow for stratification of diabetes risk include age at seroconversion, antibody number, titer, affinity, antigen specificity, and epitope binding.[174,177] Recently, novel autoantibodies have been described, including those targeting neoantigens generated in β-cells under conditions of stress (e.g., immune stress and metabolic stress). These include antibodies against modified β-cell-derived peptides or proteins generated through stress-induced post-translational modifications, like citrullination.[178]

T-Cell Biomarkers

Current evidence suggests that T-cells are the main mediators in the pathogenesis of T1DM.[179,180] Thus, T-cell biomarkers are becoming an important component of immunotherapy trials in T1DM, identifying logical targets for intervention, providing novel insights into why (or in whom) treatments succeed or fail, and providing potential for participant stratification.[181]

CD4 T-cells provide help, cytokines, and regulation, while CD8 T-cells produce inflammatory cytokines to drive the destructive immune response forward and kill β-cells.[183] Furthermore, frequencies of islet antigen-reactive CD4 and CD8 T-cells are higher in T1DM patients compared with healthy subjects, though variable over time, and may have the potential to function as a prognostic marker for disease onset or treatment response.[183,184]

The role of T cells as essential cellular constituents of disease progression has motivated research consortium efforts to develop T-cell biomarkers in T1DM, with attention to two

broad classes of markers, namely, (1) antigen specific (i.e., captured by assays that measure the number and/or function of T cells specific for B cell autoantigens) and (2) antigen agnostic (i.e., involving assays that measure T cell attributes without accounting for the specificity conferred by the T cell receptor).[185]

Conclusion and Future Perspectives

There is a vital need for the identification of more sensitive and precise biomarkers for subset of individuals with different underlying pathogenesis and difference in speed of disease progression at its earliest that will help to facilitate personalized prediction, prevention, and treatment of diabetes mellitus. Biomarkers are needed to guide our understanding of the disease process whether it is destruction of β-cells by the immune system as in T1DM or reduction in β-cell function as in T2DM. Combining biomarkers in a clinical setting may provide better sensitivity and specificity in predicting and preventing the disease. However, long-term prospective studies are needed to further to establish the utility of these biomarkers in establishing early diagnosis and management of the disease and thereby preventing complications. Furthermore, genetic studies, assessing mutations, will also provide additional insight into its association with metabolic dysregulation.[186] Different omics platforms—genomics, metabolomics, proteomics, and microbiomics—and RNA sequencing-based studies, coupled with novel data science methods involving bioinformatics, data mining, imaging, machine learning, neural networks, are now revolutionizing biomarker development.[187-189] Adoption of such methods in accordance with data protection and ethical guidelines will improve quality of life for the patients and enable better health care.

Conflict of Interest

None declared.

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