Research Progress on Early Molecular Diagnosis Methods for Type 1 Diabetes Mellitus (T1DM)

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Abstract: Type 1 diabetes mellitus (T1DM) is a chronic disease with a common symptom of hyperglycemia, which is caused by the loss of pancreatic beta-cells, leading to insulin defi-ciency. Recently, there has been much research regarding a wide range of detection methods for T1DM. The research methods involve fields such as immunology, epigenetics and microbi-ology. With more and more biomarkers being detected, earlier diagnosing markers appear more in researchers' eyes. But none of them have nearly 100% specificity and effectiveness, and they also do not fit completely with the general pattern. In this review, the writer refers to methods such as general biomarkers, immunology, and genetics and discusses them. The writ-er gets clear attributes and limitations for each method and objectively evaluates them for guid-ance in future research direction. Much further research can be done to get a clearer under-standing of newly discovered biomarkers and come up with more assertive detection methods like combination testing.

1 INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic disease with a common symptom of hyperglycemia, which is caused by the loss of pancreatic beta-cells, leading to insulin deficiency. The peak morbidity of T1DM is at the beginning of adolescence, but the incidence in adulthood is increasing attributed to the expansion of human lifespan (Vanderniet et al., 2022). The most common type of T1DM is autoimmune T1DM, defined by the body's immune system mistakenly attacking its own organs, which is the pancreas in this case. Nowadays, T1DM already has a well-accepted way of treating it – insulin injection. This can effectively control the blood glucose level in the patient's body, but it still has limitations: the insulin dosage cannot be adjusted accurately based on the real-time glucose level, especially when there are extreme blood sugar fluctuations happened commonly in the late stage. So, accompanied by multiple other factors, including severe complications if T1DM has developed like microvascular damage, early detection and management of the disease is needed in order to prevent them. For instance, identifying T1DM before the symptom starts can hugely decrease the risk of having diabetic ketoacidosis from 60% to less than 5% (Simmons & Sims, 2023).

Mănescu et al. pointed out that the majority of biomarkers currently studied and validated are late indicators, which have limited benefit for controlling the progression of the disease. Also, many reviews indicate the prevalence of researching prediction methods for T1DM is in stages 1 and 2: after seroconversion and islet autoimmunity, but if the disease can be diagnosed before stage 1, the focus can be moved on to prevent it rather than delay or treat it, also implying the significance of predicting the incidence as early as possible (Mănescu et al., 2024). Much research recently regarding the early detection of T1DM will not only provide more accurate ways of diagnosis but also have the potential to discover the disease when stage 0 to enable the avoidance of the disease. The methods of early detection of T1DM include the monitoring of related biomarkers, like GAD and IA-2 antibodies; immune cells, like CD8+ T cells and B cells; and gene expressions, like HLA genes.

In this article, the author is going to review the recent progression of the molecular diagnosis of T1DM in the early stage, including the methods mentioned above, to help clarify the future direction of research and to provide future research with reference.

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2 BIOMARKERS

1.1 Beta Cell-Related Biomarkers

1.1.1 GAD Antibodies

Glutamic acid decarboxylase (GAD) antibody (GADA) is an easy tool to detect T1DM. GAD decarboxylates glutamic acid to produce gamma amino butyric acid(GABA), which is an inhibitory neurotransmitter that suppresses glucagon secretion on pancreatic alpha cells during hyperglycemic states. Additionally, GABA acts as a growth factor on beta cells and also promotes the conversion from alpha cells to beta cells. One isoform of GAD, GAD65/GAD2, is present mostly in the pancreatic beta cells. While GADA binds with GAD65 in beta cells, beta cells will be damaged, and insulin production will be reduced (Keshavarzi et al., 2022).

According to the Finnish Type 1 Diabetes Prediction and Prevention Study (DIPP), they found biomarkers like GADA are present in people's serum before their diagnosis for an average of 1.5 years, even in the first year of some of their lives. GADA will remain increasing after T1DM pathogenesis initiates, and finally declines at the late stages. Using enzyme-linked immunosorbent assay (ELISA), GADA detection has a sensitivity of 60.8% and specificity of 100%, and T1DM diagnosis has 100% of the positive predictive value. These properties give GADA a crucial role in the diagnosis of the disease (Keshavarzi et al., 2022). In Japan, GADA is prescribed as the first biomarker when diagnosing T1DM. However, only 90% of people with T1DM will be detected with GADA, while other autoantibodies are positive in results, so multiple autoantibody testing is important for the accuracy of diagnosing T1DM (Kawasaki, 2023).

1.1.2 IA-2 Antibodies

Insulinoma-associated protein 2 (IA-2), also known as islet cell autoantigen 512 (ICA512), is a transmembrane protein found at insulin secretory granule membranes in pancreatic beta cells. Its role is to regulate the content inside the insulin secretory granule and to help with the growth of beta cells. When cytotoxic T cells initiate the autoimmune attack on islet cells in the pancreas, IA-2 antibodies and other antibodies such as GADA will appear. In the studies performed in Colorado from 1993 to 2006, Finland from 1994 to 2009, and Germany from 1989 to 2006, they are the signs of 100% risk of T1DM during the whole lifespan and can be observed readily at stage 1(categorized by The Juvenile Diabetes Research Foundation, American Diabetes Association, and Endocrine Society) of T1DM when autoimmunity starts with normoglycemia (Kawasaki, 2023).

1.1.3 C-peptide

Connective peptide (C-peptide) is an amino acid sequence that connects the A chain and the B chain of the proinsulin together. The preproinsulin synthesized in the ribosomes of pancreatic beta cells goes through several further modification processes in the granular endoplasmic reticulum, Golgi bodies, and clathrin-coated secretory granules with convertases 1 and 2; with their help, a preproinsulin will be finally converted to an insulin molecule and a C-peptide residue, stored in the secretory granules of beta cells then secreted to the blood. The amount of C-peptide secreted is equal to the moles of insulin; although there is clearance of it by the kidney and liver, the amount is too small and can be neglected. Thus, individuals with T1DM are normally observed with C-peptide levels below the normal range (0.2~0.4 nmol/L, dependent on body weight) (Maddaloni et al., 2022).

Additionally, the proinsulin/C-peptide ratio shows the potential of telling a lot of information: a study of non-diabetic twins of parents with insulin-dependent diabetes shows that they have a high value of this ratio due to insulin processing abnormalities caused by pancreatic beta cell dysfunction. But more researches are needed to confirm its reliability (Maddaloni et al., 2022).

1.2 Metabolic Biomarkers

1.2.1 Insulin and Blood Glucose

Insulin is a protein hormone that is secreted by the pancreatic beta cells, and it plays an extremely crucial role in glucoregulation. More specifically, it helps to lower the body's glucose level by promoting its uptake into muscle and adipose tissue in the form of glycogen or to be oxidized. Also, insulin has significant effects on lipid and protein metabolism. So, a lower-than-normal insulin level might be suspected as T1DM. If the disease progresses, absolute insulin deficiency might result. This can be measured by ELISA using blood, plasma, serum, or saliva (Wolkowicz et al., 2020).

Glucose is the monosaccharide that is involved in multiple metabolic pathways in the body. The hyperglycemia, a condition with excess glucose concentration in the bloodstream, might be an indicator of T1DM as well. This can be measured by Amperometry using capillary blood (Wolkowicz et al., 2020).

Simultaneous detection of insulin and glucose can now be accomplished by using self-monitoring chip-based point-of-care devices and continuous glucose monitoring (CGM) devices easily (Wolkowicz et al., 2020).

But these two can only be detected when the destruction of beta cells occurs, and the damage is irreversible. Furthermore, these symptoms are not limited to T1DM, which is not good for treating the disease compared to more early detecting methods.

3 IMMUNOLOGICAL METHODS

3.1 Immune Cells

A class of T cells with CD8 surface markers with the main function of directly killing infected and tumor cells is called CD8+ T cells. They are also known as cytotoxic T cells which release cytotoxins and produce cytokines to do their function. For the pathogenesis of T1DM, autoimmune T cells will recognize the autoantigens on beta cells presented by Antigen-Presenting Cells (APCs), and then convert into effector T cells in a very quick process. CD8+ T cells are the main contributor to the destruction of islet beta cells. CD8+ T cells are present commonly among all the human pancreas, but for people with diabetes, their amount might increase. The specific type of autoreactive CD8+ T cells that appear in most T1DM patients is preproinsulin-reactive CD8+ T cells, suggesting preproinsulin might be the primary target in the early development of T1DM (Yang et al., 2024).

Some researches recently suggested that autoantibodies involved in T1DM are not pathogenic: the primary reason for T1DM development is autoimmune T cell responses (Herold et al., 2024).

3.2 B Cells

B cells are white blood cells that produce antibodies and help with immunological memory, but as mentioned above, B cells do not contribute to the progression of T1DM much by secreting autoantibodies to destroy islet beta cells. They actually act as APCs that selectively recognize insulin, which will promote the development of disease in non-obese diabetic mice. The anergy of high-affinity insulin-binding B cells is observed to be lost both before and at the time of T1DM diagnosis. It is worth noting that people diagnosed with diabetes before the age of 7 have a common situation with a high number of B cells infiltrating the pancreatic islet cells and a fewer number of beta cells remaining compared to the people diagnosed at an older age (Herold et al., 2024).

Since T1DM is an autoimmune disease, T cells and B cells work together, that initiate and worsen the disease. As they are from the immune system of human bodies, it makes T1DM harder to cure unless using more invasive measures, but it could also bring considerable risks to the patient.

4 GENE EXPRESSION AND EPIGENETICS

4.1 Related Genes

4.1.1 HLA Genes

HLA (Human Leukocyte Antigen) is a group of proteins found on the surface of the cell that associate the presentation of exogenous and endogenous peptides to T-cell receptors, indicating that they are also a part of the major histocompatibility complex (MHC). Specifically, HLA class I gene is responsible for encoding the proteins that present endogenous peptides, and HLA class II gene is responsible for encoding the proteins that present exogenous peptides. After antigen, HLA molecules, and T cell receptors bind, they release a signal to initiate the T cell response targeting the antigenetic peptide in the beta cells. Thus, the effector function will begin and contribute to T1DM progression. The risk factors of T1DM are varied, and it is very complex to predict the risk due to diverse data sets, which are hard to conclude with a common pattern. Haplotype of HLA-DR3-DQ2.5 is commonly linked with T1D but with various strengths of association, as well as conserved HLA haplotypes such as B8-DR3 and A1-B8-DR3. There is also a very high-risk heterozygous genotype with as large as 45% of people with the risk of T1DM in some studies: DR3/DR4 (Noble, 2024). Overall, predicting T1DM using the genetic method is quite complicated as the pattern is not consistent due to large population numbers with high heterogeneity

and multiple alleles, even though some of them are also varied across the population.

Also, to help with the prediction of T1DM, using high-throughput gene expression profiling such as microarrays and RNA sequencing can help with identifying early biomarkers and tracking the disease progression by measuring the protein-coding genes' expression levels.

4.2 Epigenetics Role

4.2.1 DNA Methylation

DNA methylation refers to a biological process in which one methyl group from the S-adenosyl-L-methionine is transferred onto the C5 position of cytosines, mostly on CpG sites in mammals, and is catalyzed by DNA methyltransferases. It typically functions in transcriptional regulation/repression and maintaining genome integrity. Dysregulated DNA regulation has been observed recently in T1DM. In the genome-wide DNA methylation analysis of purified CD14+ monocytes from monozygotic twin pairs with different T1DM onset, researchers have identified 132 T1DM-methylation variable positions such as HLA-DQB1 and GAD2. They are strong evidence as they can be found at an early stage before the diagnosis and symptomatic T1DM. Further findings contribute to proving the implications of DNA methylation on the pathogenesis of T1DM (Zhang et al., 2021).

4.2.2 miRNAs

micro RNA (miRNA) is a type of small non-coding RNA (ncRNA) that regulates gene expression post-transcriptionally by binding to the 3'-untranslated region(UTR) of target mRNA transcripts, resulting in mRNA cleavage or repression of productive translation. In the case of T1DM, miRNAs affect the autoimmune initiation, beta cell dysfunction, and apoptosis. miR-98, miR-23b, and miR-590-5p have been shown to be overexpressed in CD8+ T cells from patients with T1DM. By trying to block these miRNAs, the incidence rate of T1DM can be effectively controlled (Zhang et al., 2021). This can also provide insight for future treatment of T1DM, but efficiency and feasibility are waiting to be tested.

5 DISCUSSION

This study highlights the potential of molecular biomarkers like C-peptides, several antibodies, insulin, glucose, etc. in the early detection of T1DM. Higher presentation and overexpression of most factors above will be suspected to the development of T1DM, while the under presentation of factors like C-peptide will also imply the risk of T1DM. Early detection methods are varied, but ideally, they have to be as early as possible since the destruction of islet beta cells is nearly irreversible, attributed to their complex structure and still limited understanding of the full picture of this disease. Insulin and glucose levels have significant restrictions to assist the early detection and mitigation of the disease in prior as they are symptomatic signs. Their attributes based on mechanism and understanding of the disease make them be found after mostly other biomarkers and signs mentioned in this article. For antibodies and genes, their heterogeneities among the population make their accuracy to be low, which could lead to a delayed diagnosis that causes exacerbation of the disease and delayed management. So, some scientists suggest that multiple tests at the same time might be a solution to this problem, but more work should be done to test their efficiency and figure out the best combination of testing that can be used in future diagnoses medically. A good thing is that more and more genes and ncRNAs are discovered to have a relation with T1DM, but this also indicates that a wide range of effort should be put in to discover more of their mechanism, application, and so on.

6 CONCLUSION

In the recent five years, there has been huge progress in research regarding the measures of T1DM early detection. Methods ranging from immunology to epigenetics to microbiology are devoted to more accurate and reliable prediction and detection of T1DM, especially in the early stages, which enables people to manage the disease prior to its exacerbation. This article provides various reviews regarding the mechanisms of ways of diagnosis. The writer discussed their limitations and evaluated their future directions of research by summarizing the emerging biomarkers discovered and initially proved to have the potential associated with detection. In this article, not all the detection methods and biomarkers are listed and discussed, and some of them are not rich in

detail. Combination detection seems to have strong potential in future applications, so more research is needed to develop this method, and then, the pattern researchers will get can contribute to the finding of the best and most general way of combination testing.

REFERENCES

Herold, K. C., Delong, T., Perdigoto, A. L., Biru, N., Brusko, T. M., & Walker, L. S. K. 2024. The immunology of type 1 diabetes. Nature Reviews. Immunology, 24(6), 435–451.

Kawasaki, E. 2023. Anti-Islet autoantibodies in Type 1 diabetes. International Journal of Molecular Sciences, 24(12), 10012.

Keshavarzi, E., Noveiry, B. B., & Rezaei, N. 2022. The relationship between GAD65 autoantibody and the risk of T1DM onset. Journal of Diabetes & Metabolic Disorders, 21(2), 1935–1942.

Maddaloni, E., Bolli, G. B., Frier, B. M., Little, R. R., Leslie, R. D., Pozzilli, P., & Buzzetti, R. 2022. C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective. Diabetes Obesity and Metabolism, 24(10), 1912–1926.

Mănescu, M., Mănescu, I. B., & Grama, A. 2024. A review of Stage 0 biomarkers in Type 1 diabetes: the holy grail of early detection and prevention? Journal of Personalized Medicine, 14(8), 878.

Simmons, K. M., & Sims, E. K. 2023. Screening and prevention of Type 1 diabetes: Where are we? The Journal of Clinical Endocrinology & Metabolism, 108(12), 3067–3079.

Vanderniet, J. A., Jenkins, A. J., & Donaghue, K. C. 2022. Epidemiology of Type 1 diabetes. Current Cardiology Reports, 24(10), 1455–1465.

Wolkowicz, K. L., Aiello, E. M., Vargas, E., Teymourian, H., Tehrani, F., Wang, J., Pinsker, J. E., Doyle, F. J., Patti, M., Laffel, L. M., & Dassau, E. 2020. A review of biomarkers in the context of type 1 diabetes: Biological sensing for enhanced glucose control. Bioengineering & Translational Medicine, 6(2).

Yang, K., Zhang, Y., Ding, J., Li, Z., Zhang, H., & Zou, F. 2024. Autoimmune CD8+ T cells in type 1 diabetes: from single-cell RNA sequencing to T-cell receptor redirection. Frontiers in Endocrinology, 15.

Zhang, J., Chen, L., Zou, Y., Zhang, S., Xiong, F., & Wang, C. 2021. Implication of epigenetic factors in the pathogenesis of type 1 diabetes. Chinese Medical Journal, 134(9), 1031–1042.