Diabetes, Metabolic Syndrome and Obesity

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REVIEW

Diabetic Retinopathy and Cardiovascular Disease: A Literature Review

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Abstract: Diabetic complications can be divided into macrovascular complications such as cardiovascular disease and cerebrovascular disease and microvascular complications such as diabetic retinopathy, diabetic nephropathy and diabetic neuropathy. Among them, cardiovascular disease (CVD) is an important cause of death in diabetic patients. Diabetes retinopathy (DR) is one of the main reasons for the increasing disability rate of diabetes. In recent years, some studies have found that because DR and CVD have a common pathophysiological basis, the occurrence of DR and CVD are inseparable, and to a certain extent, DR can predict the occurrence of CVD. With the development of technology, the fundus parameters of DR can be quantitatively analyzed as an independent risk factor of CVD. In addition, the cytokines related to DR can also be used for early screening of DR. Although many advances have been made in the treatment of CVD, its situation of prevention and treatment is still not optimistic. This review hopes to discuss the feasibility of DR in predicting CVD from the common pathophysiological mechanism of DR and CVD, the new progress of diagnostic techniques for DR, and the biomarkers for early screening of DR.

Keywords: diabetic retinopathy, cardiovascular disease, pathophysiological mechanism, optical coherence tomography angiography, biomarkers

Introduction

Diabetic complications can be divided into macrovascular complications such as cardiovascular disease (CVD) and cerebrovascular disease and microvascular complications such as diabetic retinopathy (DR), diabetic nephropathy and diabetic neuropathy. Diabetes and its complications are posing a huge threat to human health. DR is an important cause of blindness among working-age people.[1] About one fifth of diabetic patients in the world suffer from DR, and the number of patients with vision loss caused by DR will continue to rise.[2] CVD is one of the leading causes of death, and the number of deaths increased by 14.5% between 2006 and 2016 globally.[3]

DR can be divided into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) with or without macular edema (DME).[4] PDR is the late stage of DR and its main pathophysiological changes include thickening of retinal capillary basement membrane, increased retinal vascular permeability, tissue ischemia and hypoxia, and neovascularization. Because the neovascularization are very fragile, it is prone to penetration, which can lead to vitreous hemorrhage and retinal detachment, ultimately resulting in vision loss.[5]

Cardiovascular disease is still the leading cause of death in diabetic patients, and in recent years, some studies have found that DR is closely related to the occurrence of CVD and can predict the occurrence of CVD.[6,7] This may be due to the fact that DR and CVD share common risk factors such as hyperglycemia and hypertension, as well as pathophysiological mechanisms, including common oxidative stress, inflammation, and epigenetic modifications.[8–13] However, there is currently no consensus on the feasibility of using DR to predict CVD. This review discusses the feasibility of DR in predicting the occurrence of CVD from the aspects of common risk factors and pathophysiological mechanisms of DR and CVD, new advances in diagnostic DR technology, and biomarkers for early screening of DR, hoping to provide new ideas for the prevention and detection of cardiovascular diseases (Figure 1).

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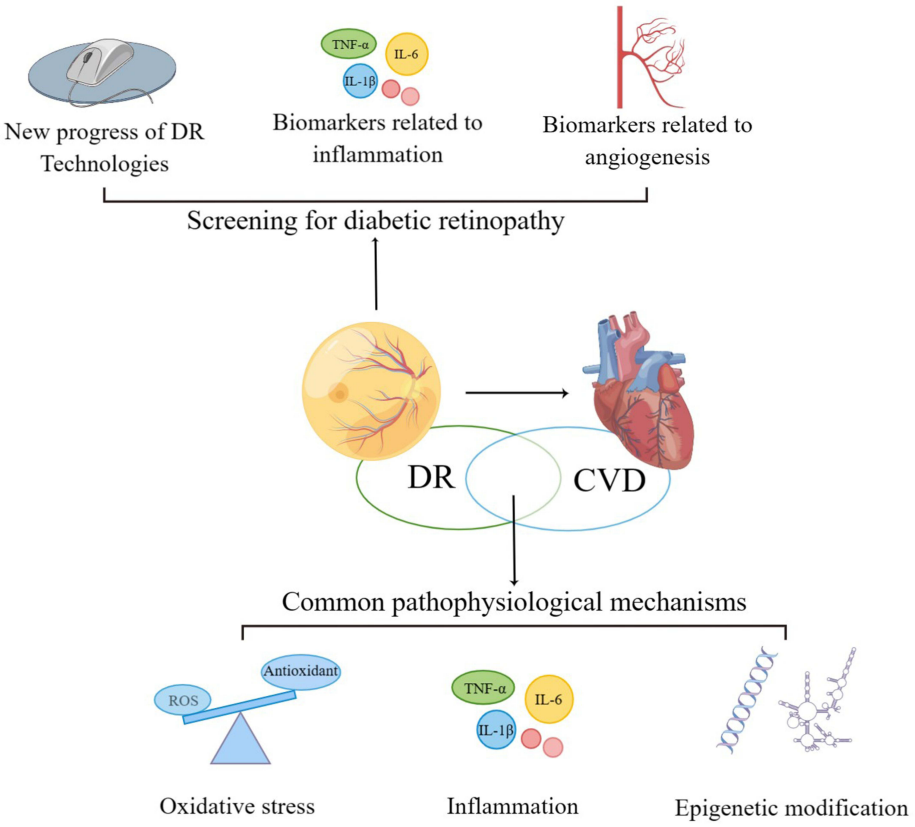

Figure 1 The main content of the review (By Figdraw). This review discusses the feasibility of DR in predicting CVD from the common risk factors and pathophysiological mechanisms of DR and CVD, the new progress of diagnostic techniques for DR, and the biomarkers for early screening of DR.

Common Risk Factors for DR and CVD

DR and CVD are common vascular complications of diabetes, and the occurrence and development of vascular complications in diabetic patients are related to factors such as age of onset, course of disease, hyperglycemia, hypertension, abnormal lipid metabolism and obesity.[14,15] Therefore, factors such as hyperglycemia, hypertension and obesity can be intervened to prevent the occurrence of DR and CVD. A prospective study showed that strengthening the control of blood glucose in patients with diabetes can significantly reduce the incidence of microvascular complications including diabetic retinopathy, but there is no significant reduction in the risk of cardiovascular disease.[16] However, over time, the effect is cumulative, and continued follow-up for 10 years found that the relative risk of microvascular disease continued to decrease, and the risk of cardiovascular disease was significantly reduced.[17] Diabetes with hypertension increases the risk of CVD and DR in patients with diabetes,[15,18] and the study results show that strict control of blood pressure can significantly reduce the risk of CVD and DR in patients with diabetes.[19] High BMI is one of the strongest risk factors for diabetes, and is associated with many metabolic abnormalities that lead to insulin resistance. Changing unhealthy diets and lifestyles is an effective way to address obesity and prevent complications of diabetes.[14]

Common Pathophysiological Mechanism of DR and CVD

Oxidative Stress

Studies have shown that the unified mechanism of diabetic microvascular and macrovascular complications may be an excess superoxide produced by the mitochondrial electron transport chain induced by hyperglycemia.[8] Oxidative stress occurs when the production of reactive oxygen species (ROS) and the body's endogenous antioxidant defense mechanisms are out of balance, damaging cells in target organs such as the heart and retina.[20] DR and CVD are common

microvascular and macrovascular complications of diabetes, oxidative stress has been proved to be one of the key factors leading to diabetic complications.

The abnormalities of the four metabolic pathways are related to the microvascular and cardiovascular complications of diabetes induced by oxidative stress, which are the activation of protein kinase C (PKC) pathway, the increase of polyol pathway flux, the activation of hexosamine pathway and the accumulation of advanced glycation end products (AGEs).[21] Four abnormal metabolic pathways caused by hyperglycemia can lead to the increase of vascular endothelial growth factor (VEGF), Endothelin-1 (ET-1), transforming growth factor-β (TGF-β) and Insulin-like growth factor-1 (IGF-1), which in turn causes angiogenesis, vascular infiltration, blood-retinal barrier (BRB) damage, and ultimately result in DR.[22] Metabolic abnormalities of diabetes can also promote each other with oxidative stress, and further cause inflammatory response mediated by Nuclear factor-kappa B (NF-κB), which eventually leads to the occurrence and development of DR.[22] Metabolic abnormalities in diabetes lead to excessive production of ROS in vascular endothelial cells and myocardium.[23] In addition, insulin resistance causes excessive ROS production by mitochondria in large vascular endothelial cells by increasing the flux of Free fatty acids (FFA) from adipocytes to arterial endothelial cells and oxidation.[8] Excessive production of ROS may react with the NO produced by endothelial nitric oxide synthase (eNOS) to generate peroxynitrite, which in turn can uncouple eNOS and make it lose its anti-atherosclerotic activity.[24] eNOS promotes the production of NO in blood vessels, and NO induces vascular smooth muscle relaxation through the activation of guanylate cyclase (GC) and the formation of cyclic guanosine monophosphate (cGMP) to dilate blood vessels.[25] At the same time, NO can also increase the concentration of cGMP in platelets, resulting in the inhibition of intracellular Ca^{2+} level, and finally inhibit platelet aggregation.[26] The functional manifestation of eNOS uncoupling is endothelial dysfunction, which is an early marker of most cardiovascular diseases.[27] In addition, ROS can directly inactivate another key anti-atherosclerotic enzyme, prostacyclin synthase (PGIS).[23] PGIS is the catalyst of prostacyclin synthesis. Prostacyclin can inhibit platelet aggregation and vasoconstriction, and PGIS gene has been proved to be related to cardiovascular disease[28] (Figure 2).

Inflammation

Inflammation is a non-specific response of tissue to various injury stimuli. More and more evidence suggests that inflammation plays an important role in the occurrence and development of DR and CVD. Chronic low-grade inflammation exists in all stages of DR. Endothelial cells of diabetic retina increase the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which leads to retinal leukocyte aggregation and the release of various cytokines, leading to low-grade inflammation.[9] Inflammatory mediators destroy the tight junctions between endothelial cells, increase vascular permeability, and destroy BRB, leading to the occurrence

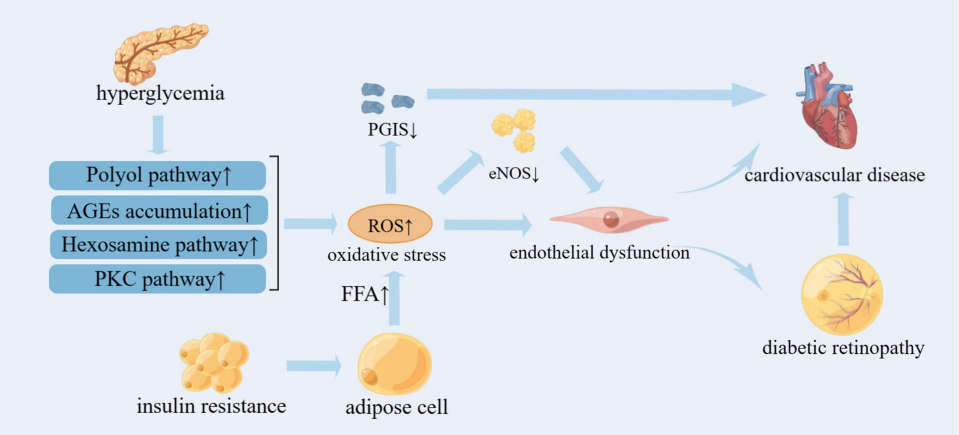

Figure 2 The role of oxidative stress on DR And CVD (By Figdraw). Hyperglycemia induces the activation of PKC pathway, the increase of polyol pathway flux, the activation of hexosamine pathway and the accumulation of AGEs, which promote oxidative stress. In addition, insulin resistance increases the flux and oxidation of FFA from adipocytes into arterial endothelial cells, which leads to excessive ROS production in mitochondria. Excessive ROS production directly leads to endothelial dysfunction or indirectly leads to endothelial dysfunction by uncoupling eNOS, and further induces DR and CVD.

of DME.[29] Metabolic abnormalities caused by hyperglycemia lead to excessive production of ROS in mitochondria, increase oxidative stress, further activate NF-κB, and induce the up-regulation of inflammatory cytokines, and eventually lead to increased vascular permeability and further aggravate DR.[8] The main pathological feature of PDR is neovascularization. Pro-inflammatory cytokines can bind with target endothelial cells to directly induce angiogenesis, or indirectly participate in angiogenesis by stimulating endothelial cells to produce proangiogenic mediators.[30] On the contrary, angiogenic factors such as VEGF and Angiopoietin-1 (Ang-1) can also up-regulate the expression of inflammatory cytokines and cause pro-inflammatory response in endothelial cells.[30]

Inflammation is a common pathophysiological basis for atherosclerosis, which is the pathological basis of CVD. Unstable atherosclerotic plaque rupture, platelet aggregation and thrombosis will eventually lead to CVD.[10,11] Leukocyte recruitment and pro-inflammatory cytokines are characteristics of the early stage of atherosclerosis, which is considered to be blood flow-mediated inflammatory changes of endothelial cells (ECs),[31] and when ECs are activated by inflammation, the expression of various inflammatory factors increases, such as monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), ICAM-1, VCAM-1 and so on, attracting lymphocytes and monocytes that bind to the endothelium and infiltrate the arterial wall,[32] among which VCAM-1 seems to play a major role. When VCAM-1 adheres to the activated ECs, monocytes will penetrate between intact endothelial cells under the interaction of various chemokines, especially MCP-1 and its receptor CCR2. So far, monocytes have acquired the characteristics of tissue macrophages, combined with oxLDL, resulting in foam cells[33] and foam cells secrete inflammatory cytokines, amplifying the local inflammatory response of the lesion site.[34] Matrix metalloproteinases (MMPs) can degrade collagen fibers in the extracellular matrix of plaques. In advanced atherosclerosis, a large number of macrophages play a key role in plaque rupture, bleeding and thrombosis by secreting MMPs.[35]

In the presence of hyperglycemia, diabetic retinopathy may be driven by inflammation, resulting in uncontrolled synthesis of inflammatory mediators by endothelial cells, which leads to a surge of inflammatory mediators entering the systemic circulation, triggering the development of atherosclerosis, and eventually causing the occurrence of cardiovascular disease[36] (Figure 3).

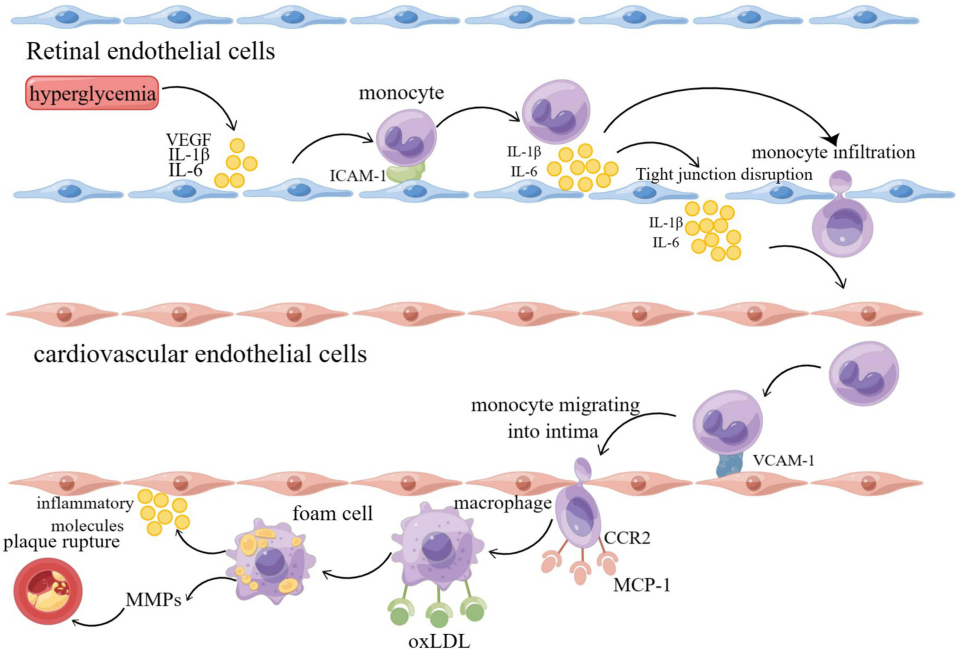

Figure 3 Hypothesis of inflammation as a key factor in the development of atherosclerotic cardiovascular disease induced by diabetic retinopathy (By Figdraw). Hyperglycemia causes retinal endothelial cell dysfunction and produces inflammatory factors such as VEGF and IL-6. Inflammatory factors destroy the tight junction between endothelial cells, destroy BRB, promote the extravasation of inflammatory cells such as monocytes, and further aggravate the inflammatory state of tissues. Driven by inflammation, endothelial cells synthesize uncontrolled inflammatory mediators, resulting in a surge of inflammatory mediators entering the systemic circulation. When vascular endothelial cells are activated by inflammation, it leads to the development of atherosclerosis and eventually leads to the occurrence of CVD.

Epigenetic Modification

Epigenetic modification changes gene expression without affecting DNA sequence, and is an interaction between gene and environment. Epigenetic modification has been found to regulate oxidative stress, inflammation and angiogenesis in diabetes.[37] DNA methylation, histone modification and non-coding RNA are important subtypes of epigenetic modification, which can affect gene transcription and regulate diabetic vascular complications.[12,13,38]

DNA Methylation

DNA methylation is the earliest discovered and the most important epigenetic modification. Under the catalysis of DNA methyltransferase (DNMTs), s-adenosine-methionine (SAM) is methylated at the 5'- position of cytosine residues, and this modification mainly occurs in rich regions of CpG island.[39] DNA methylation is involved in the occurrence of diabetic vascular complications in many ways.[40] It is well known that cardiovascular disease is the main cause of death in patients with diabetes. Studies have shown that hypomethylation occurs in atherosclerotic lesions, and this is associated with increased transcriptional activity.[41,42] For example, during hyperglycemia, DNA hypomethylation of the $p66^{Shc}$ promoter in human aortic endothelial cells leads to overexpression of $p66^{Shc}$, inducing oxidative stress and thereby accelerating endothelial dysfunction.[43] In atherosclerosis, plaques develop preferentially in arterial regions with disturbed blood flow (d-flow). D-flow controls epigenomic DNA methylation patterns in a DNMT-dependent manner, which in turn alters endothelial gene expression and induces atherosclerosis.[44] In addition, DNA methylation also occurs in several genes related to the pathogenesis of atherosclerosis, such as eNOS, hypoxia-inducible factor-α (HIF-1α) and MMPs.[45] Other CVD risk factors, such as hyperhomocysteinemia, hypercholesterolemia and inflammation, are also associated with atherosclerosis-related DNA methylation.[46] Other studies have shown that the DNA demethylase TET2 prevents atherosclerosis by inhibiting the upregulation of proinflammatory cytokines and chemokines and the activation of inflammasomes.[47] This indicates that TET2 is a promising therapeutic target for the treatment of atherosclerosis. DR is another common vascular complication of diabetes, and DNA methylation plays a key role in different pathogenesis of DR, including oxidative stress, inflammation and neovascularization.[48–50] ROS can activate DNMTs and promote DNA methylation by deprotonating cytosine molecules.[51] On the other hand, DNA methylation promotes oxidative stress and ultimately contributes to the development of DR.[52] Hyperglycemia can also inhibit DNMT1 recruitment to the matrix metalloproteinase-9 (MMP9) promoter region, resulting in an increase in MMP9, thus accelerating the inflammatory response of diabetic retinopathy.[52] Maternal expression gene 3(MEG3) is a kind of lncRNA that can promote apoptosis and is widely expressed in eye tissues. MEG3 can effectively inhibit retinal neovascularization by down-regulating the expression of phosphatidylinositol 3-kinase (PI3K), serine / threonine kinase (AKT), VEGF and pro-inflammatory factor.[53] Studies have shown that DNMT1 inhibits MEG3 expression by promoting MEG3 promoter methylation, thus accelerating endothelial-mesenchymal transformation (EndMT) in diabetic retinopathy,[54] and promoting the proliferation, migration and angiogenesis of human retinal microvascular endothelial cells (hRMEC),[55] In addition, DNA methylation also plays a role in other diabetic vascular complications, such as decreased methylation in the promoter region of the pro-inflammatory circulating protein ANGPTL2, which promotes the occurrence and development of albuminuria in patients with type 2 diabetes.[56]

Histone Modification

Histone methylation refers to the transfer of methyl groups from S-adenosine-L-methionine to lysine or arginine residues in histones by histone methyltransferases (HMTs). Lysine can be monomethylated, dimethylated or trimethylated, while arginine can be monomethylated, symmetrical or asymmetrically dimethylated.[57] Histone methylation of specific lysine or arginine residues plays a key role in the occurrence of diabetic vascular complications.[58] For example, aberrant histone methylation (H3K4me1, H3K9me2 and H3K9me3) at the promoters of NADPH oxidase (Nox4) and eNOS leads to continuous up-regulation of these two genes, which increases ROS production and further leads to endothelial dysfunction.[59] Alkemade et al demonstrated that the level of H3K27me3 in VSMC decreased in apoE $^{-/-}$ rats.[60] Another study confirmed that the overall H3K9me2 and H3K27me2 were significantly reduced in atherosclerotic lesions.[61] Similarly, lower levels of H3K27Me3 were found in blood vessels in advanced atherosclerotic plaques without changes in the corresponding histone methyltransferase EZH2.[62] In macrophages, JMJD3, a specific H3K27me3

demethylase, can regulate H3K27me3 levels in response to inflammation and transcriptional activity by binding to PcG. When exposed to lipopolysaccharide (LPS), the level of JMJD3 in macrophages increases and the inflammatory response is enhanced.[63] This suggests that H3K27 demethylation is essential for atherosclerotic plaque formation. Protein arginine methyltransferase 4 (PRMT4) specifically catalyzes H3R17 methylation to regulate apoptosis of retinal pigment epithelial cells induced by oxidative stress.[64] Hyperglycemia increases the level of H3K27me3 in human retinal endothelial cells and retinal microvessels of diabetic rats, activates Ezh2 in diabetes, and promotes the recruitment of enzymes responsible for regulating DNA methylation of MMP-9 promoter, which result in transcriptional activation and enhanced inflammatory response.[65]

Histone acetyltransferase (HATs) and histone deacetylase (HDACs) mediate histone acetylation and deacetylation, respectively. HATs use acetyl-CoA as a cofactor to catalyze the transfer of acetyl to the ε-amino group of the lysine side chains, thus reducing the binding of histone to DNA and activating gene expression. HDACs reverse lysine acetylation and repress gene expression.[57] Studies have shown that HATs may participate in the pathophysiology of diabetic microvascular complications by regulating the expression of inflammatory pathway genes. For example, RelA/p65, a subunit of NF-κB, induces NF-κB activation through mutual regulation of acetylation and deacetylation, which promotes inflammation.[66,67] The H3 acetylation of TNF-α and COX-2 promoters in human blood monocytes increased in type 1 and type 2 diabetes subjects.[68] Hyperglycemia leads to histone acetylation in the retina, which contributes to the upregulation of pro-inflammatory proteins induced by hyperglycemia, thereby promoting the development of diabetic retinopathy.[69] OxLDL induces acetylation of IL-8 and chemokine MCP-1 promoter by recruiting p300 in endothelial cells, and oxLDL reduces the expression and binding affinity of HDAC1 and HDAC2, thus triggering inflammation and promoting atherosclerosis.[70] In addition, hyperglycemia-induced superoxide overactivation is considered to be the main pathway of diabetic vascular complications,[71] while ROS have been shown to increase HDAC activity and decrease HAT activity, as well as inhibit histone acetylation.[72] It has been reported that lysine acetyltransferase 1 (KAT1) is significantly down-regulated in the retinal tissue of model mice, which promotes neovascularization and vascular leakage in mouse retinal tissue.[73] Extracellular superoxide dismutase (SOD) is an antioxidant enzyme that protects vascular cells from oxidative stress. Exendin 4, a glucagon-like peptide-1 receptor agonist, induces the expression of SOD in human retinal microvascular endothelial cells through histone H3 acetylation.[74]

Non-Coding RNA

Non-coding RNA (ncRNAs) is mainly divided into micro-RNA (miRNAs), long non-coding RNA (lnc RNAs) and circular RNA (circ RNAs), which play a transcriptional regulatory role in diabetic vascular complications.[75]

MiRNA is a highly conserved small ncRNA of 20~40 nucleotides. The generation of miRNA first occurs in the nucleus, where the gene that encodes miRNA is transcribed into pri-miRNAs by RNA polymerase. Subsequently, the pri-miRNA is processed by RNase III in the nucleus to form pre-miRNA. In the cytoplasm, the pre-miRNA is transported to the cytoplasm by the exportin5 protein, and then it is cleaved by another RNase III to form mature miRNA. The mature miRNA binds to the RNA-induced silencing complex (RISC) to block protein translation or induce gene degradation by binding to the 3'-untranslated region of the target mRNA at the post-transcriptional level to regulate gene expression.[76,77] The discovery of miRNA has shown that the non-histone coding region of the genome contains important information about life activities, which not only plays an important role in the normal development and physiology of organisms, but also participates in the pathological process of multiple diseases, including diabetes, cancer, cardiovascular disease and autoimmune diseases.[78,79] MiRNAs has also been shown to contribute to vascular complications of diabetes. For example, miRNA-200b is down-regulated in retinal endothelial cells and cardiac microvascular endothelial cells exposed to high glucose, and its down-regulation leads to VEGF overexpression and EndMT, which is associated with the development of diabetic retinopathy and cardiovascular diseases.[80,81] Circulating levels of miR-146a are decreased in diabetic patients.[82] MiR-146a reduces endothelial inflammatory response induced by high glucose by inhibiting the expression of Nox4,[83] decreases the expression of interleukin-1 receptor-associated kinase-1 (IRAK-1) and VCAM-1/ICAM-1,[84] and reduces the transmission of TLR4 / NF-κB and TNF-α signal pathway.[85]

LncRNA is a class of ncRNA with a length of more than 200bp, which is thought to be involved in a variety of biological processes, such as epigenetic regulation, transcription, translation, splicing and cell differentiation.[86] For example, LncRNA metastasis-associated lung adenocarcinoma transcript 1(MALAT1) has been extensively studied in diabetes. MALAT1 can promote ECs proliferation and retinal neovascularization.[87] MALAT1 has also been found to promote cardiomyocyte proliferation by activating the phosphoinositide kinase (PI3K) / protein kinase B (PKB) signal pathway.[88] Up-regulated expression of MALAT1 was found in ApoE$^{-/-}$mice fed with high-fat diet, and overexpression of MALAT1 enhanced the effect of ox-LDL on EndMT in human umbilical vein endothelial cells (HUVEC), indicating that MALAT1 was involved in the development of atherosclerosis.[89] In addition, MALAT1 can damage vascular endothelial cells by activating the NF-κB signal pathway and inducing diabetic inflammatory factors such as IL-6, TNF-α and IL-1β,[90] Generally speaking, MALAT1 plays a role in both DR and CVD.

CircRNAs are closed circular RNAs, which are produced by reverse splicing of specific regions of heterogeneous nuclear RNA.[91] CircRNAs mainly regulate the occurrence of diabetic vascular complications through the miRNA-mRNA axis. For example, circRNA (CircR)-284 is reported to promote atherosclerosis by targeting inhibition of micro-RNAs (miRs)-221, while CircR-284 increases the risk of plaque rupture.[92] The down-regulation of circRNA DMNT3B increases the expression of miR-20b-5p and promotes the proliferation, migration and angiogenesis of human retinal microvascular endothelial cells (HRMECs).[93] Under the condition of HG, circ_001209 is overexpressed in HRVECs, which indirectly regulates the expression of COL12A1 by down-regulating miR-15b-5p, resulting in vascular endothelial cell dysfunction.[94] However, the epigenetic modification of circRNA-miRNA-mRNA axis in DR and CVD is still limited and needs further study.

In summary, epigenetic modification may play an important role in the pathophysiology of diabetes and its related vascular complications, including atherosclerotic cardiovascular disease and retinopathy, and provide a unique opportunity to develop new treatments for diabetic complications.

The above is a summary of the common pathophysiological mechanisms of DR and CVD. At present, it is known that there is a certain correlation between DR And CVD, but the specific mechanism and common regulatory pathways need to be further studied.

The Predictive Effect of DR on CVD

Diabetic complications can interact with each other. For example, chronic kidney disease can be detected from retinal images, which provides the feasibility of using retinal photography to screen for chronic kidney disease in the community population.[95] Carotid atherosclerosis parameters can predict the outcome of microvascular and cardiovascular complications in patients with type 2 diabetes.[96] Many studies have suggested that diabetic retinopathy can predict the occurrence of cardiovascular disease, and patients with diabetic retinopathy have an increased risk of cardiovascular disease. Studies have found that there is a close relationship between microvascular and macrovascular complications of diabetes. A clinical study on type 2 diabetic retinopathy found that DR is associated with an increased incidence of CVD after adjusting for risk factors such as age, gender, blood pressure, smoking status, and total cholesterol/high-density lipoprotein ratio, and DR is an independent predictor of CVD.[97] A 10-year follow-up study found that functional and structural retinal microvascular changes can predict cardiovascular events in patients with type 1 diabetes.[98] Therefore, non-invasive imaging of retinal microvessels can be performed to detect changes in microvessels and to predict the occurrence of cardiovascular events.[99] In addition, whether serological biomarkers, which measure the severity of DR, can be used for early warning of cardiovascular events is also an interesting topic. Figure 4).

New Progress of Screening DR Technologies

The traditional imaging examinations of DR mainly include fundus color photography and fundus fluorescein angiography (FFA). FFA is extremely important for evaluating the clinical fundus characteristics of DR, and has important guiding significance for the staging, treatment guidance and prognosis of DR, which is considered as the gold standard for identifying the existence of neovascularization. However, traditional angiography techniques have the limitations of affecting vascular imaging due to contrast medium leakage, inability to image the retinal capillary network in layers, and inability to observe neovascularization directly,[100] and injection of contrast medium may bring adverse reactions such as

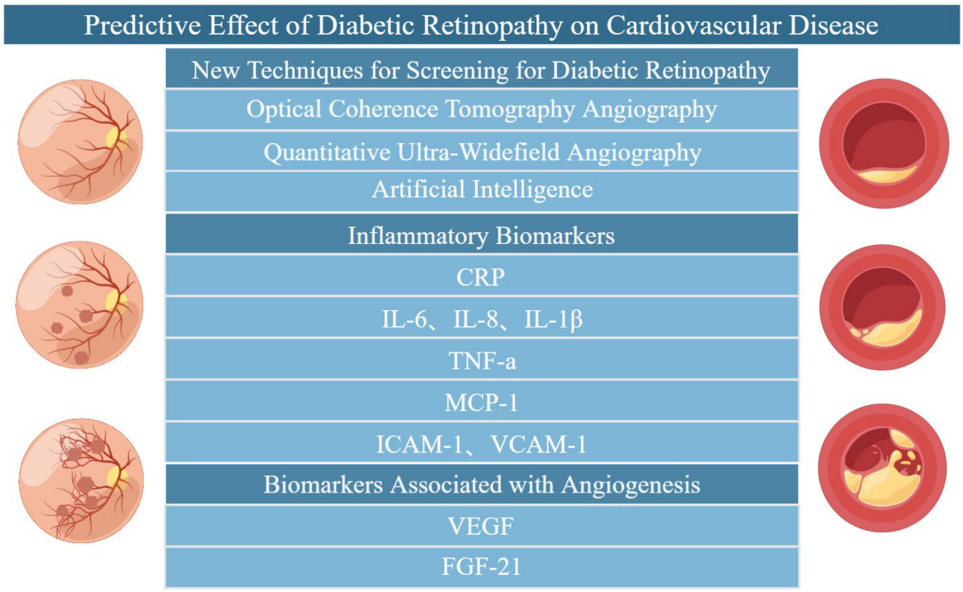

Figure 4 Predictive effect of diabetic retinopathy on cardiovascular disease (By Figdraw). Early screening of DR through the new technology of DR and related serum biomarkers to prevent the occurrence of atherosclerotic cardiovascular disease.

nausea and vomiting. The emergence of optical coherence tomography angiography (OCTA) breaks the deadlock, and its high-resolution and non-invasive characteristics enable in-depth analysis of a series of pathophysiological changes of DR.[101] Compared with FFA, OCTA is more accurate in evaluating capillary non-perfusion, but its ability to detect microaneurysms is lower than FFA.[102] Meanwhile, OCTA can better detect the formation of early retinal neovascularization.[103]

The grading of the severity of DR is very important for the diagnosis, treatment and prognosis evaluation of the disease, and the warning of CVD through DR is also inseparable from the quantitative analysis of the severity of DR. In recent years, researchers have been trying to determine vascular changes in DR and whether they can inform the development of CVD. A prospective study of DR predicting CVD risk found that the addition of retinal microvascular parameters greatly improved CVD risk prediction.[104] With the development of ophthalmological instruments, OCTA can quantitatively analyze the retinal capillary microvasculature of diabetic patients in a non-invasive manner.[105] The common parameters for quantitative analysis of retinal microvessels are perfusion density and vessel density.[106–108] OCTA has the potential to show retinal capillary perfusion and its control.[106] Studies have shown that the perfusion index of retinal vessels may be a useful biomarker for judging the severity of DR.[107] Another study shows that the vascular density measured by OCTA is related to the severity of DR, which may guide the stage of DR.[108] In addition, vessel diameter index and fractal dimension are also visible quantitative parameters. Studies have found that there is a negative correlation between the severity of DR and the vessel density and fractal dimension, and a positive correlation with vessel diameter index.[109]

Early microvascular damage can also be detected by OCTA in patients with subclinical diabetic retinopathy.[110] Compared with healthy controls, patients with subclinical DR have microvascular changes in the superficial and deep capillary plexus, as shown by increased foveal vessel density, parafoveal and foveal ischemic zone (FAZ) area.[111] Therefore, OCTA is an effective tool for early screening of DR.

In addition to OCTA, the severity of DR can also be evaluated by using quantitative ultra-widefield angiography indicators such as leakage index, ischemic index, and microaneurysm count.[112]

In recent years, with the development of information technology and the scientific progress of big data, the research of artificial intelligence (AI) has made unprecedented progress. Methods based on machine learning (ML), especially deep learning (DL), can not only identify, locate and quantify the pathological features of DR, but also diagnose or classify DR stages.[113] In addition to identifying DR, AI recognition of retinal fundus images is also possible to predict

cardiovascular risk factors that were previously thought to be absent or unquantifiable in retinal images, such as age, sex, smoking status, and systolic blood pressure.[114] Although AI has certain limitations in clinical diagnosis and management and cannot completely replace ophthalmologists, recently, fully automated systems based on AI have been further developed and preliminarily approved for DR screening,[115] and it is believed that AI will play an important role in DR Screening in the near future.

Serum Biomarker

Inflammation and angiogenesis play an important role in the occurrence and development of DR. Various pathological changes in diabetic patients can up-regulate the expression of VEGF, which can lead to angiogenesis and activate various inflammatory mediators,[116] At the same time, inflammatory mediators can also induce the expression of VEGF.[117] Changes in the concentrations of various pro-inflammatory and angiogenic mediators were found in the serum of patients with DR, which are related to the severity of DR and can be used as biomarkers of the severity of DR.

Biomarkers Related to Inflammation

As mentioned above, chronic low-grade inflammation exists at every stage of DR, and many studies have confirmed that inflammatory mediators can be used as biomarkers to measure the severity of DR and predict the progress of DR. C-reactive protein (CRP) is produced in liver and adipose tissue and is largely regulated by IL-6.[118] It has been found that the concentration of serum CRP is significantly increased in DR patients and positively correlated with the severity of DR.[119] TNF-α can promote leukocyte siltation, increase the production of ROS, and promote the destruction of BRB.[120] It has been reported that the level of serum TNF-α is highly correlated with PDR and can be used as an independent inflammatory marker of PDR.[121] Shimizu et al also found that serum IL-6 concentration was significantly correlated with the severity of DME and may also be a predictor of PDR.[122] In addition, the levels of serum inflammatory factors such as IL-1β and IL-8 are also reported to be related to DR stages.[123] However, other studies have shown that there is little association between inflammatory markers and diabetic retinopathy after controlling for established risk factors including duration of diabetes, A1C, systolic blood pressure, waist-to-hip ratio, and use of diabetes medications.[124] Due to the uncertainty of the results, the clinical value of serum inflammatory mediators as DR biomarkers should be reconsidered. Furthermore, regional concentrations of inflammatory mediators in the retina may be more meaningful than the serum levels of inflammation that reflect the systemic effects of diabetes. IL-1, IL-6, IL-8, TNF-A, MCP-1, ICAM-1 and VCAM1 were found to be elevated in the vitreous of diabetic patients with DR, and were related to the pathogenesis of DR.[125–128] It is worth noting that although inflammatory levels in diabetic retinopathy are generally considered to be chronically low, the levels of IL-8 and MCP-1 in vitreous fluids are comparable to those found in the pleural effusion of patients with pneumonia or tuberculosis.[129] It has also been reported that the levels of inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α in aqueous humor reflect the severity of DR and are related to prognosis.[130] Moreover, a prospective study observed that TNF-α levels in tears were highly correlated with the severity of DR.[131] It has also been reported that inflammatory biomarkers such as IL-6 and TNF-α in saliva are also associated with the severity of DR, suggesting that these salivary biomarkers are potential biomarkers for predicting the progression of DR.[132]

Biomarkers Related to Angiogenesis

VEGF is considered to be the main angiogenic growth factor related to the development of DR.[133] The VEGF family consists of seven members, namely VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF (placental growth factor), of which VEGF-A is the prototype of the VEGF family and is therefore sometimes referred to simply as VEGF. Hyperglycemia induces ischemia and hypoxia, oxidative stress, and overactivation of PKC, which eventually leads to various VEGF-mediated pathological processes, such as angiogenesis, increased endothelial permeability, reduced pro-apoptotic protein inhibition and disruption of vascular homeostasis.[116] VEGF increases the levels of VCAM-1, ICAM-1 and MCP-1 through NF-κB -mediated pathway, and increases leukocyte adhesion, which further aggravates endothelial dysfunction.[134,135] Ahuja et al measured the level of serum VEGF in healthy control group, non-DR group, NPDR group and PDR group, and found that it showed a significant increasing trend, which is a reliable biomolecule biomarker to judge the severity of DR.[136] A meta-analysis conducted by Zhou et al yielded the same

results.[137] Similarly, VEGF levels in the vitreous are significantly associated with DR.[138] Through a cross-sectional study, Ang et al found that the level of VEGF in tears of patients with DR was correlated with the severity of DR.[139] Fibroblast growth factor 21 (FGF-21) also has proangiogenic effects, and Lin et al found that serum FGF21 levels were independently associated with the severity of DR.[140]

In summary, biomarkers related to the pathogenesis of DR can predict the severity of DR, but their serum levels may come from systemic effects, so levels of cytokines in vitreous and aqueous humor may be more meaningful. Compared with aqueous humor, vitreous, tears and saliva are more easily obtained and noninvasive, so the levels of cytokines in tears and saliva may be more promising tools for screening and predicting DR. However, these results need to be confirmed in larger studies.

Conclusion

The prediction of cardiovascular risk in people with diabetes can carry out targeted preventive treatment for asymptomatic patients who are at high risk of developing diabetes. The severity of DR can be used to predict the occurrence of CVD, which may be because diabetic retinopathy and cardiovascular disease are in the same pathophysiological environment, in which oxidative stress, inflammation and epigenetic modification seem to play a key role, but its specific mechanism needs to be further studied. Early screening of DR by fundus imaging to prevent CVD may be feasible, but the new technology is not mature and has not been widely implemented. In addition, the related serum biomarkers are not highly specific and can not accurately reflect the pathological degree of DR. Therefore, more specific markers of vitreous, aqueous humor and tears are needed to prove their significance.

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Disclosure

The authors report no conflicts of interest in this work.

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