Circulating Biomarkers of Diabetic Retinopathy: An Overview Based on Physiopathology

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Received 25 December 2015; Accepted 18 May 2016

Abstract

Diabetic retinopathy (DR) is the main cause of working-age adult-onset blindness. The currently available treatments for DR are applicable only at advanced stages of the disease and are associated with significant adverse effects. In early stages of DR the only therapeutic strategy that physicians can offer is a tight control of the risk factors for DR. Therefore, new pharmacological treatments for these early stages of the disease are required. In order to develop therapeutic strategies for early stages of DR new diagnostic tools are urgently needed. In this regard, circulating biomarkers could be useful to detect early disease, to identify those diabetic patients most prone to progressive worsening who ought to be followed up more often and who could obtain the most benefit from these therapies, and to monitor the effectiveness of new drugs for DR before more advanced DR stages have been reached. Research of biomarkers for DR has been mainly based on the pathogenic mechanism involved in the development of DR (i.e., AGEs, oxidative stress, endothelial dysfunction, inflammation, and proangiogenic factors). This review focuses on circulating biomarkers at both early and advanced stages that could be relevant for the prediction or detection of DR.

1. Introduction

Diabetic retinopathy (DR) is the most frequent complication of diabetes and the main cause of blindness in working-age adults in the developed countries [1]. DR prevalence in the diabetic population is around one-third, with one-tenth having vision-threatening states such as diabetic macular edema (DME) or proliferative diabetic retinopathy (PDR) [2]. Moreover DR entails considerable costs related to both treatment and social support [3, 4]. As the disease remains asymptomatic until the pathology is significantly advanced, screening to detect it during the early stages is necessary [5].

The actual available treatments for DR are applicable only at advanced stages of the disease and are associated with significant adverse effects [6–8]. In early stages the only therapeutic strategies that physicians can offer are a tight control of the risk factors for DR. The principal risk factors for developing DR are hypertension, glycemic control, and diabetes duration [9–20]. However, clinical studies in diabetic patients reveal a substantial variation in the onset and severity of DR [21–24], thus indicating that genetic factors may influence the susceptibility to developing DR [25].

In order to develop new therapeutic strategies for early stages of DR new diagnostic tools are urgently needed. In this regard, circulating biomarkers could be useful (i) to detect early disease, (ii) to identify diabetic patients most prone to progressive worsening, in whom intensified therapy could be prioritized, and (iii) to monitor the effectiveness of new drugs for DR before advanced DR stages have developed.

A biomarker has been defined as “a biological molecule found in blood, or other bodily fluids, or tissue which represents a sign of a normal or abnormal process of a condition or disease.” Therefore, “a biomarker may be used to see how well the body responds to a treatment for a disease or condition” [26, 27]. Biomarkers may help to identify people with subclinical disease and also to monitor the clinical disease [28], for example, to assess treatment response. Ideally, a biomarker has to be measured in accessible tissues [28]. As
the retina constitutes a small proportion of total body weight, a circulating biomarker for DR should be highly specific to the retina rather than a marker of systemic vascular disease.

Research of biomarkers for DR has been based on the pathogenic mechanism involved in the development of DR. In this review we will summarize the more important molecules that could become biomarkers for DR.

2. Advanced Glycation End Products

The nonenzymatic glycation reaction is known to be one of the most significant mechanisms contributing to tissue damage seen in diabetes. It involves a complex series of chemical reactions that lead to the formation of early glycation products, alpha-dicarbonyls, which are directly toxic to both tissues and precursors of AGEs (advanced glycation end products). AGE accumulation contributes to diabetic complications through direct tissue damage as well as through the activation of specific AGE receptors (RAGE) [29–32].

Several AGEs have been proposed as biomarkers for DR. N-Epsilon-carboxymethyl lysine (N-ε-CML), the most abundant of circulating AGEs, has been extensively studied. N-ε-CML has been found elevated in the serum of diabetic patients and to an even higher extent in those with microvascular complications [33–37]. Interestingly, Choudhuri et al. [38] found that subjects with nonproliferative diabetic retinopathy (NPDR) showed a significantly higher level of N-ε-CML compared to the PDR group. Secondly, pentosidine is an AGE that has also been related to DR, and some studies found its blood levels elevated in patients with PDR compared with NPDR or patients without DR [35, 36, 39]. However, in the EURODIAB study [40] the association between pentosidine and DR disappeared after controlling for diabetes duration. As in the case of N-ε-CML, Salman et al. [41] detected a significant elevation of pentosidine levels in patients during the earliest detectable phase of DR (early NPDR) and more elevation at the preproliferative stage, returning to lower levels at the proliferative stage of DR. These findings suggest that both pentosidine and N-ε-CML can be used as a biochemical marker for the early occurrence of DR and as a warning factor in the preproliferative stage of DR. More prospective studies are needed to confirm these findings, with the glomerular filtration rate (GFR) being taken into account, because AGEs tend to increase with the reduction of the GFR [36]. Additionally, skin collagen pentosidine and N-ε-CML levels, measured in human skin punch-biopsies samples, also predicted the progression of DR in two prospective studies [42, 43].

Highly reactive dicarbonyl compounds such as 3-deoxyglucosone (3-DG) have been identified as important intermediates of the glycation reaction, not only as precursors of AGEs but also for their direct effects in cell functions [44, 45]. Kusunoki et al. [46] found that 3-deoxyglucosone levels were higher in diabetic patients than in control subjects and further increased according to the severity of the DR. As previously mentioned, some of the nonenzymatic glycation consequences are due to AGEs binding their receptor (RAGE). Soluble RAGE blood levels are low in patients with diabetic complications in comparison with diabetic patients without complications and nondiabetic subjects [37]. By contrast, increased levels of this receptor in patients with PDR compared with those with NPDR or without DR have also been reported [47, 48].

RAGEs activation induces both permeability of microvascular endothelial cells and production of reactive oxygen species (ROS) [49, 50]. Choudhuri et al. [38] found that ROS in peripheral blood mononuclear cell (PBMC ROS) increased significantly in NPDR and PDR subjects compared to diabetic patients without DR and control subjects. This increase strongly correlated with N-ε-CML. Some authors have proposed N-ε-CML as the key molecule for triggering the production of ROS, leading, for example, to lipid peroxidation and oxidative DNA damage [51]. Malondialdehyde (MDA), which is a metabolite produced during phospholipid peroxidation, is increased in subjects affected with PDR compared to those affected with NPDR or healthy controls [51, 52], further suggesting that oxidative stress and lipid peroxidation are involved in the DR progression. Further data is required to establish the role of soluble RAGE, PBMC ROS, and MDA as possible biomarkers of DR.

3. Biomarkers of Basement Membrane and Extracellular Matrix Turnover

DR is associated with important disturbances in the structure and metabolism of basement membranes [53–55]. Basement membrane thickening and increased vascular permeability are two major retinal vascular changes associated with the pathogenesis of DR [56–58]. Collagen IV and laminin are components of the basement membranes [59] and have been proposed as biomarkers of DR.

Collagen IV is a major matrix protein of the basement membranes, and elevated serum and urine levels have been associated with diabetic microangiopathic complications, especially DN [60–64]. Lee et al. [59] found that plasma levels of 7S-collagen, a collagen IV domain resistant to various proteases, are elevated in DR and DN and that its levels increased with the severity of both diseases. The authors also found that diabetic patients without complications had higher levels of this domain than healthy subjects. Kotajima et al. [65] also found higher levels in serum and also in the vitreous fluid of diabetic patients with DR than in those without this disease. However there are few prospective studies evaluating the role of collagen IV as a DR biomarker and most of them had a relatively small number of subjects.

Laminin is a noncollagenous glycoprotein of basement membranes which is upregulated by high glucose concentrations [66, 67]. It has been postulated that serum levels of this protein, or its fragments, could reflect the changes observed in the basement membranes of diabetic patients. Although the association between laminin-P1 concentrations, the largest pepsin resistant fragment of laminin, and DN seems established [68–72], the results concerning DR are less clear. In this regard, whereas Pietschmann et al. [68] and Hayashi et al. [60] found no correlation between DR and laminin-P1, our group has established a positive correlation between laminin-P1 and DR in a diabetic population [73]. In addition, we demonstrated that panretinal photocoagulation significantly reduced the increased serum Lam-P1 levels detected in
diabetic patients [74]. This was certainly intriguing, given that retinal microcirculation represents only a minor part of the total number of blood vessels in the body. Moreover, our findings suggest that the contribution of the Lam-P1 produced in the retina to its circulating levels is significant and that serum Lam-P1 levels could be a useful biomarker for DR. In a prospective study we also found that circulating laminin-P1 was not a useful predictor of DR development (at least over a 4-year period) but that it was an early marker of the presence of DR, as well as a marker of its severity [75].

Metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade extracellular matrix proteins and are modulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). In DR the balance between MMPs and TIMPs is impaired and increased levels of MMP-9 and MMP-2 are found in the retina and vitreous. MMPs appear to play a multiple role, being proapoptotic, proinflammatory, and proangiogenic, and MMP-9 seems to act as a modulator of inflammation in the pathophysiology of DR [76, 77]. Furthermore, increased MMPs may be implicated in the disruption of the blood retinal barrier (BRB), an early event in the development of DR [77].

MMP-9 is the largest and the most complex member of the MMP family and TIMP-1 shows a greater preference for MMP-9 [77]. Jacqueminet et al. [78] found that type 1 diabetic patients have both higher circulating levels of MMP-9 and a higher MMP-9/TIMP-1 ratio. In the same study, patients affected with DR showed elevated systemic MMP-9 and an elevated MMP-9/TIMP-1 ratio compared to patients without DR. Similarly, Béranek et al. [79] found both MMP-2 and MMP-9 elevated in plasma of PDR patients [80]. Recently, in the EURODIAB Prospective Complication Study, based on a cohort of type 1 diabetic subjects, plasma MMP-2 levels showed a significant positive association with the development of PDR at 6–9 years of follow-up. It should be emphasized that this study was prospective and that the analyses were adjusted for cardiovascular risk factors and HbA1c [81].

4. Biomarkers Related to Inflammation

A large body of evidence supports the role of inflammation mediators in the pathogenesis of DR [82–86]. Some of the molecules implicated in both systemic and local inflammation have been tested as possible biomarkers of DR. The EURODIAB Prospective Complication Study hypothesized that a Z-score composed by C-reactive protein (CRP), Tumor Necrosis Factor-α (TNF-α), and interleukin-6 (IL-6) could be associated with the presence of vascular complications in diabetic patients. They found a positive correlation between these inflammatory factors and DR, DN and cardiovascular disease (CVD) [40]. In this section, we will focus on the possible role of these inflammatory molecules as biomarkers of DR.

CRP belongs to the pentraxin family of calcium-dependent ligand-binding proteins and is produced in the liver in response to IL-6. It is an acute-phase protein and a marker of inflammation and tissue damage [87]. CRP has been associated with macrovascular disease and with DN [88, 89]. Some studies have found a positive association between CRP levels and the prevalence of DR, in both type 1 and type 2 diabetic patients [90–93]. By contrast, other authors have found no relationship between CRP and DR [94–98] or with its progression [99]. However, these conflicting results could be due to some confounding factors that were not taken into account. For example, in the EURODIAB study [40], the association between CRP and DR decreased after adjustment for the body mass index (BMI). In fact, the BMI is intimately related to CRP levels. Lim et al. [100] in a population-based, cross-sectional study of 718 persons with diabetes in the Singapore Malay Eye Study (SiMES) reported that diabetic patients with higher levels of CRP and a higher BMI were less likely to have DR. The authors propose that this protective effect could be due to its proangiogenic properties, which may be beneficial in the preproliferative stages of DR, and also due to its anti-inflammatory effects. Further studies would help determine the possible protective effect of CRP in DR.

IL-6 has been found elevated in the vitreous fluid of diabetic patients with DR and has been implicated in the pathogenesis of DR [101, 102]. Higher levels of systemic IL-6 were detected in diabetic children with DR than in those without DR [103]. Moreover, Shimizu et al. [104] found that serum IL-6 concentration correlates significantly with the severity of macular edema and could be a predictor of PDR. However, these results have not been confirmed in larger studies [95, 96, 98].

TNF-α is a cytokine that promotes the irreversible adhesion of leukocytes to the endothelium (leukostasis), increases the production of reactive oxygen species, and is implicated in BRB breakdown [105, 106]. A strong correlation between plasma levels of TNF-α and PDR has been reported [98, 107]. Klein et al. [97] reported that this correlation was mediated by the presence of kidney disease. In children, Zorena et al. [108] found that the risk of NPDR was strongly dependent on TNF-α levels. Finally, it has been reported that baseline circulating TNF-α is a predictor of DR incidence [109] as well as of the progression of diabetic complications [110]. Interestingly, it has been observed that the TNF-α level in tears is highly correlated with DR severity [111].

4.1. Adhesion Molecules. The adhesion molecules are implicated in leukostasis and some of them act as angiogenic factors. Leukostasis plays an important role in diabetic vascular leakage, capillary nonperfusion, and endothelial cell damage [85]. Adhesion molecules are also markers of endothelial dysfunction [112]. The most important are intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, and these are found in high concentrations in the vitreous of patients affected with PDR [113–116]. Matsumoto et al. [117] found that ICAM-1 was associated with the presence of DR, but not with macroangiopathy, while E-selectin and VCAM-1 were associated with both micro- and macroangiopathy complications. VCAM-1 has been associated with the presence of DR [97, 118, 119], and both VCAM-1 and E-selectin have been associated with the presence of DR, DN, and CVD [120]. On the other hand, in the Hoorn study [121], a Z-score combining CRP and ICAM was found to be associated with the presence of DR. Moreover, Roy et al. [109] found in a prospective study in
an African-American population that baseline ICAM-1 levels were associated with the incidence of DME and that baseline E-selectin levels were associated with DR progression. In addition, Spijkerman et al. [99] in a prospective study found that baseline E-selectin levels were also associated with DR progression, whereas VCAM-1 was not significantly associated with the presence or progression of DR. However, none of these findings have been supported by other studies [98, 122–124]. Notably, Ügurlu et al. [125] assessed the levels of ICAM-1 and VCAM-1 in early stages of DR (when a biomarker is much needed) and found no differences between patients with or without DR.

5. Miscellaneous Candidate Proteins

5.1. Retinol-Binding Protein 4. Retinol-binding protein 4 (RBP4) is a transport protein for vitamin A and also an adipokine, which is secreted by hepatocytes and adipose tissue [126]. Graham et al. [127] found that serum levels of RBP4 are related to insulin resistance in subjects with obesity, impaired glucose tolerance, or T2DM. Moreover, elevated levels are associated with increases in the BMI, the waist-to-hip ratio, triglyceride levels, and systolic blood pressure and with decreased high-density lipoprotein cholesterol levels; all of them are components of the metabolic syndrome. As the levels of RBP4 are inversely correlated with the expression of GLUT4 in adipocytes, it has been postulated that elevated RBP4 contributes to insulin resistance downregulating GLUT4 [128].

Inflammation plays an essential role in DR development [82, 83]. In this regard, RBP4 has been correlated not only with obesity and insulin resistance but also with inflammatory factors such as C-reactive protein and IL-6 [129]. It has been demonstrated that RBP4 induces inflammation in human retinal endothelial cells by stimulating the expression of proinflammatory molecules [130]. Moreover, in an animal model overexpressing RBP4, early-onset microglia activation followed by progressive retinal degeneration mediated by an increased expression of pro-IL-18 was observed [131]. Therefore, RBP4 could be involved in the inflammatory process of DR and could be considered a biomarker of the early stages of DR. Takebayashi et al. [132] found elevated RBP4 levels in T2DM patients in comparison with nondiabetic subjects and significantly increased levels in patients with PDR versus no DR or nonproliferative DR. However, this relation was reduced after adjusting for urinary albumin excretion (UAE). Moreover, Li et al. [133] found that both UAE and serum RBP4 levels were significantly higher in PDR patients. Nevertheless, other studies have not found any association between RBP4 levels and DR [134, 135].

There are some factors that could influence RBP4 levels such as the BMI, some drugs (i.e., antidiabetic and hypolipidemic agents), vitamin A deficiency, and the GFR [126, 132, 134]. Therefore, further research taking into account these confounding factors is needed.

5.2. Adrenomedullin. Adrenomedullin (ADM) is a peptide that was first isolated from the acid extract of human pheochromocytoma [136]. Later, it was found to act as a circulating hormone [137] produced mainly in the vascular endothelium rather than in the adrenal medulla [138]. ADM has various functions including vasodilatation, the regulation of vascular cell growth, hormone secretion, and natriuresis [139]. In the eye ADM is produced by the retinal pigment epithelium (RPE), vascular endothelial cells, fibroblasts, macrophages, hyalocytes, and glial cells [140]. ADM has been related with the pathophysiology of many vitreoretinal disorders and inflammatory retinal diseases and its plasma levels have been found to be higher in patients with diabetes [141]. In addition, elevated levels of ADM have been reported in the vitreous fluid from patients with DR [142, 143], as well as in retinal membranes of PDR patients [143]. Notably vitreous ADM correlated with the prognosis of this disease [143].

Elevated plasma levels of ADM are found in patients with DR compared to controls and diabetic patients without DR [144, 145]. However, in a group of children and adolescents with T1DM without fundoscopic alteration but with functional abnormalities in the ERG examination, ADM levels were not increased [146]. These findings suggest that circulating levels of ADM are not sensitive enough to detect neurodegeneration and that more advanced stages of DR are needed to induce a significant increase in ADMA plasma levels.

5.3. Homocysteine. Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine [147, 148]. This molecule is considered to be a risk factor for cardiovascular disease, although the exact mechanism by which homocysteine causes atherosclerosis is little known. Experimental evidence suggests that homocysteine produces an endothelial dysfunction through ROS, decreases the production of endothelial-derived nitric oxide, stimulates vascular smooth-muscle cell proliferation, increases the formation of highly atherogenic oxysterols and lipid peroxidation, has a thrombogenic effect [147], and has a stimulatory effect on the expression of VEGF [149].

Although some studies, mostly performed in type 2 diabetic patients, have established an association between plasma levels of homocysteine and DR [148, 150, 151], especially with PDR [149, 152–155] or DME [156, 157], these findings have not been confirmed in other studies [158, 159]. Moreover, in a recent study [149] an association between plasma homocysteine and vitreous homocysteine in PDR was found, the increased vitreous levels being attributed to BRB breakdown. Further experimental studies are needed to establish the role of homocysteine in the pathogenesis of DR.

The level of homocysteine is higher in males and increases with age, renal impairment, the use of certain drugs (i.e., metformin, phenytoin, and methotrexate), and the nutritional deficiency of vitamin cofactors (i.e., folate, vitamin B12, and vitamin B6) required for homocysteine metabolism [148, 149, 160]. Therefore, these factors should be taken into account when the relationship between homocysteine levels and the presence of DR is being analyzed. In this regard, case-control studies adjusted for the major risk factors for DR and for determinants of homocysteine concentrations, including vitamin B12 and folate levels, have shown a positive association between homocysteine and DR [150, 160]. As mentioned above, renal disease is a potential confounding
factor. Pepys and Hirschfield [87] found an association between homocysteine and vision-threatening DR, but this was no longer significant after adjustment for serum creatinine and the UAC (urine albumin creatinine) ratio. However, in other studies the association persisted after adjusting for renal function [150, 156, 157, 160].

In conclusion, homocysteine could be a biomarker of DR, but most of the studies have demonstrated a relationship in advanced stages of the disease. Further studies taking into account possible confounding factors such as renal function are required.

5.4. ADMA. Endothelial dysfunction and impaired ocular hemodynamics underlying DR development are associated with decreased nitric oxide (NO) synthase activity and NO bioavailability, thus resulting in vasoconstriction and increased ROS. Serum asymmetric dimethylarginine (ADMA) is involved in the NO pathway and serum levels of ADMA have been found elevated in diabetic patients with DR [161–163].

The exact mechanism leading to the elevation of the ADMA level has not been clarified, but decreased activity of dimethylarginine dimethylaminohydrolase (DDAH), which metabolizes ADMA to citrulline and dimethylamine, has been implicated because DDAH is inactivated by ROS [164, 165]. Notably, ADMA has a NO synthase-independent action that upregulates ACE (angiotensin-converting enzyme) expression and also promotes vasoconstriction and vascular thickening in eNOS knock-out mice [166].

6. Endothelial Progenitor Cells (EPCs)

EPCs are marrow-derived cells involved in adult neovascularization and endothelial homeostasis and are known to be stimulated by several modulators such as VEGF, erythropoietin, and substance P [167–169]. EPCs are increased in PDR [170–173], especially in mature forms [173]. On the other hand, some authors found a decreased level of EPCs in NPDR [172, 173], but these findings were not confirmed in other studies [171].

It has been postulated that low levels of EPCs in peripheral blood contribute to macrovascular diabetic complications [174, 175] while an increase of these levels is related to DR. Fadini et al. [170] called this phenomenon the “diabetic paradox” and suggested that an increase of growth factors and/or cytokines contributes to preferential homing of EPCs to the retina rather than to diabetic hearts or limbs. In this regard, Zerbini et al. [176] found that one of the EPCs, the CFU-Hill cells, which are positive for both endothelial markers and hematopoietic and monocytic lineage markers, manifests abnormalities in association with the presence of NPDR and possibly before clinical evidence of retinopathy. These abnormalities result in a reduced expression of adhesion molecules, which may have consequences for facilitating the progression of retinopathy. Moreover, Tan et al. [177] found that EPCs were impaired in their ability to migrate and to repair damaged capillary endothelium in PDR patients.

7. Immunocomplexes and Autoantibodies

It has been postulated that humoral factors have a possible role in the pathogenesis of vascular complications [178, 179]. Indeed, immunocomplexes (IC) and anti-cardiolipin antibodies of the IgM type are found in higher levels in diabetic patients with vascular complications [179]. However, few studies have specifically addressed their potential role as a biomarker of DR.

In diabetes, damage to the BRB enables extravasation and subsequent lipoprotein modification, which are sufficient to initiate the synthesis of autoantibodies that, in turn, lead to the formation of IC. These IC in DR have been associated with pericyte loss, leukostasis, the activation of macrophages, and the stimulation of growth factors. Lopes-Virella et al. [180, 181] reported the association of IC containing advanced glycation end product- (AGE-) LDL (AGE-LDL) and oxidized LDL (oxLDL) with the presence and progression of DR.

On the other hand, some antibodies have been also associated with DR. Li et al. [182] in in vitro studies found that retinal pericyte-reactive antibodies induced cellular damage by activating complement. In addition, anti-pericyte antibodies have been found in T2D patients especially during earlier stages of DR, probably due to a reaction with antigens expressed by “activated” pericytes [183, 184]. In advanced stages of DR the prevalence of antibodies declines and this could mark pericyte loss and disease progression [184]. Moreover, antibodies against CD38 (antigen that is present on retinal pericytes) have been found in the serum of diabetic patients [185–188]. Further studies are needed to elucidate the role of these antibodies as biomarkers in DR.

8. Circulating RNA

Nucleic acids have been identified in peripheral blood, so providing a new potential tool for diagnosis and/or prognosis. It has been suggested that the concentration of plasma nucleic acids (DNA and RNA) reflects the degree of cell death. In fact, they are increased in numerous pathological processes, for example, in numerous cancers [189]. We will review the implications of circulating mRNA and microRNA as possible biomarkers for DR.

8.1. mRNA. Hamaoui et al. [190] investigated mRNA levels of rhodopsin in plasma samples from diabetic patients with different degrees of DR. Rhodopsin is a visual pigment found exclusively in the rod cells of the retina [191] and it was detected in peripheral blood of healthy individuals and diabetic patients with and without DR. With the exception of PDR, there was a trend for the levels of mRNA of rhodopsin to increase with the severity of retinopathy. In the case of the PDR group in which there were no differences with the group of healthy individuals, the authors argued that in this state the cells are exhausted in a metabolic capacity or in total number [190]. In a further study they combined the detection of mRNA from rhodopsin with mRNA from retinal amine oxidase, a protein also exclusively expressed in the retina which is found to decrease with the progression of DR, and calculated the ratio between them. The ratio had a higher area...
under the ROC curve than rhodopsin alone, which allowed them to differentiate mild from severe DR [192].

Other potential biomarkers are mRNA circulating levels of RPE65 and retinoschin, both exclusive of the retina. Shalchi et al. have demonstrated that circulating RPE65 mRNA increases with DR severity while plasma mRNA levels from retinoschin decrease [193].

The authors of these studies suggest that the possible mechanisms by which circulating mRNA levels of RPE65 and retinoschin increase with the severity of DR are release from dead and dying retinal cells and possible upregulation of their transcription and/or controlled secretion of their mRNA [190, 193]. However, it should be noted that in all of these cases the mRNA was also detected in healthy and diabetic patients without retinopathy. Thus, longitudinal studies could help explore the role as predictors of DR development.

8.2. MicroRNA. MicroRNAs (miRNAs) are endogenously produced short coding RNAs of about 20–22 nucleotides that have an important role in modulating gene expression, inhibiting the expression of their target genes via posttranscriptional mechanisms [194–196]. Due to their stability in biofluids such as blood and urine, because it is relatively easy to quantify them, and because some of them are cell-type or tissue-specific, miRNAs are potential biomarkers for the early detection of DR [197, 198]. In recent years, several miRNAs have emerged as important regulators of particular aspects of DR pathology [199]. Indeed, there are some miRNAs which have been found decreased in animal models of DR, such as miRNA-146a, miRNA-200b, and miRNA-29b, whose target genes are fibronectin (implicated in fibrosis and basement membrane thickening), VEGF (an angiogenic factor), and PAX (an activator of a proapoptotic pathway), respectively. The reduction of these miRNAs is related to overexpression of the corresponding proteins in DR [200–202]. Recently, García de la Torre et al. [203] performed a study in a group of type 1 diabetic patients measuring the expression of miR-126 in EPCs and found increased levels in those patients with DR.

However, although the tide of miRNA research is rising fast, the potential for miRNAs to act as noninvasive biomarkers and even therapeutic targets has yet to be elucidated.

9. Concluding Remarks

In order to develop therapeutic strategies for the early stages of DR new diagnostic tools are urgently needed. In this regard, circulating biomarkers could be useful not only for detecting those patients at early stages of the disease but also for identifying those patients most prone to progressive worsening. In this subset of patients the tight control of blood glucose levels and blood pressure should be prioritized.

A limiting factor in searching for biomarkers of DR is that their plasma changes can reflect systemic effects of long-standing diabetes rather than specific damage in the retina. In this regard, the studies addressed to investigate circulating levels of proteins and/or miRNAs that are exclusively expressed in the retina should be prioritized. The combination of specific markers of DR and general markers of diabetes-induced microangiopathy would appear as feasible means of advance in this research area. On the other hand, the identification of protective biomarkers is a challenge still to be met. The comparison by means of high-throughput methods of plasma samples from patients with long-standing diabetes without DR with those patients with DR could help us in identifying these “protective” proteins or metabolites.

Finally, circulating biomarkers could be useful not only for identifying the early stages of DR but also for monitoring the effectiveness of new drugs and for identifying the group of patients whose response is the most significant. Such a strategy would permit us to optimize the resources of healthcare system, but this will require the combined efforts of ophthalmologists, diabetologists, basic researches, and healthcare providers.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] N. Cheung, P. Mitchell, and T. Y. Wong, “Diabetic retinopathy,” The Lancet, vol. 376, no. 9735, pp. 124–136, 2010.
[2] J. W. Y. Yau, S. L. Rogers, R. Kawasaki et al., “Global prevalence and major risk factors of diabetic retinopathy,” Diabetes Care, vol. 35, no. 3, pp. 556–564, 2012.
[3] E. M. Pelletier, B. Shim, R. Ben-Joseph, and J. J. Caro, “Economic outcomes associated with microvascular complications of type 2 diabetes mellitus: results from a US claims data analysis,” Pharmacoeconomics, vol. 27, no. 6, pp. 479–490, 2009.
[4] E. Heintz, A.-B. Wiiréhn, B. B. Peebo, U. Rosenvist, and L.-Å. Levin, “Prevalence and healthcare costs of diabetic retinopathy: a population-based register study in Sweden,” Diabetologia, vol. 53, no. 10, pp. 2147–2154, 2010.
[5] S. Jones and R. T. Edwards, “Diabetic retinopathy screening: a systematic review of the economic evidence,” Diabetic Medicine, vol. 27, no. 3, pp. 249–256, 2010.
[6] Q. Mohamed, M. C. Gillies, and T. Y. Wong, “Management of diabetic retinopathy: a systematic review,” The Journal of the American Medical Association, vol. 298, no. 8, pp. 902–916, 2007.
[7] R. Simó and C. Hernández, “Advances in the medical treatment of diabetic retinopathy,” Diabetes Care, vol. 32, no. 8, pp. 1556–1562, 2009.
[8] R. Simó and C. Hernández, “Intravitreous anti-VEGF for diabetic retinopathy: hopes and fears for a new therapeutic strategy,” Diabetologia, vol. 51, no. 9, pp. 1574–1580, 2008.
[9] I. M. Stratton, E. M. Kohner, S. J. Aldington et al., “UKPDS 50: risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis,” Diabetologia, vol. 44, no. 2, pp. 156–163, 2001.
[10] H. C. Looker, J. Krakoff, W. C. Knowler, P. H. Bennett, R. Klein, and R. L. Hanson, “Longitudinal studies of incidence and progression of diabetic retinopathy assessed by retinal photography in Pima Indians,” Diabetes Care, vol. 26, no. 2, pp. 320–326, 2003.
[11] E. Agardh, C.-D. Agardh, S. Koul, and O. Torffvit, “A four-year follow-up study on the incidence of diabetic retinopathy
in older onset diabetes mellitus,” *Diabetic Medicine*, vol. II, no. 3, pp. 273–278, 1994.

[12] O. Cohen, K. Norymberg, E. Neumann, and H. Dekel, “Complication-free duration and the risk of development of retinopathy in elderly diabetic patients,” *Archives of Internal Medicine*, vol. 158, no. 6, pp. 641–644, 1998.

[13] Y. Yoshida, R. Hagura, Y. Hara, G. Sugawara, and Y. Akanuma, “Risk factors for the development of diabetic retinopathy in Japanese type 2 diabetic patients,” *Diabetes Research and Clinical Practice*, vol. 51, no. 3, pp. 195–203, 2001.

[14] Diabetes Control and Complications Trial Research Group, “The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial,” *Diabetes*, vol. 44, no. 8, pp. 968–983, 1995.

[15] I. M. Stratton, A. I. Adler, H. A. W. Neil et al., “Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study,” *The British Medical Journal*, vol. 321, no. 7258, pp. 405–412, 2000.

[16] B. Jerneld and P. Algvere, “Relationship of duration and onset of diabetes to prevalence of diabetic retinopathy,” *American Journal of Ophthalmology*, vol. 102, no. 4, pp. 431–437, 1986.

[17] The Diabetes Control and Complications Trial Research Group, “The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus,” *The New England Journal of Medicine*, vol. 329, no. 14, pp. 977–986, 1993.

[18] R. Turner, “Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33),” *The Lancet*, vol. 352, no. 9131, pp. 837–853, 1998.

[19] D. R. Matthews, I. M. Stratton, S. J. Aldington, R. R. Holman, and E. M. Kohner, “Risks of progression of retinopathy and vision loss related to tight blood pressure control in type 2 diabetes mellitus: UKPDS 69,” *Archives of Ophthalmology*, vol. 122, no. 11, pp. 1631–1640, 2004.

[20] “Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38,” *British Medical Journal*, vol. 317, no. 7160, pp. 703–713, 1998.

[21] S. E. Moss, R. Klein, and B. E. K. Klein, “Ten-year incidence of visual loss in a diabetic population,” *Ophthalmology*, vol. 101, no. 6, pp. 1061–1070, 1994.

[22] The Diabetes Control and Complications Trial Research Group, “Clustering of long-term complications in families with diabetes in the diabetes control and complications trial,” *Diabetes*, vol. 46, no. 11, pp. 1829–1839, 1997.

[23] A. Patel, S. MacMahon, J. Chalmers et al., “Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial,” *The Lancet*, vol. 370, no. 9590, pp. 829–840, 2007.

[24] H. A. Keenan, T. Costacou, J. K. Sun et al., “Clinical factors associated with resistance to microvascular complications in diabetic patients of extreme disease duration: the 50-year medalist study,” *Diabetes Care*, vol. 30, no. 8, pp. 1995–1997, 2007.

[25] O. Simó-Servat, C. Hernández, and R. Simó, “Genetics in diabetic retinopathy: current concepts and new insights,” *Current Genomics*, vol. 14, no. 5, pp. 289–299, 2013.

[26] Biomarkers Definitions Working Group, National Institutes of Health Director’s Omotoatoave on Biomarkers and Surrogate Endpoints, “Biomarkers and surrogate endpoints: preferred definitions and conceptual framework,” *Clinical Pharmacology & Therapeutics*, vol. 69, no. 3, pp. 89–95, 2001.

[27] National Cancer Institute at the National Institutes of Health, Dictionary on Cancer Terms, http://www.cancer.gov/dictionary.

[28] T. J. Lyons and A. Basu, “Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers,” *Translational Research*, vol. 159, no. 4, pp. 303–312, 2012.

[29] P. J. Beisswenger, “Glycation and biomarkers of vascular complications of diabetes,” *Amino Acids*, vol. 42, no. 4, pp. 1171–1183, 2012.

[30] A. W. Stitt, “The role of advanced glycation in the pathogenesis of diabetic retinopathy,” *Experimental and Molecular Pathology*, vol. 75, no. 1, pp. 95–108, 2003.

[31] A. Goldin, J. A. Beckman, A. M. Schmidt, and M. A. Creager, “Advanced glycation end products: sparking the development of diabetic vascular injury,” *Circulation*, vol. 114, no. 6, pp. 597–605, 2006.

[32] A. M. Schmidt, S. D. Yan, J.-L. Wautier, and D. Stern, “Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis,” *Circulation Research*, vol. 84, no. 5, pp. 489–497, 1999.

[33] M. P. Wautier, P. Massin, P. J. Guillausseau et al., “N(carboxymethyl)lysine as a biomarker for microvascular complications in type 2 diabetic patients,” *Diabetes and Metabolism*, vol. 29, no. 1, pp. 44–52, 2003.

[34] B. O. Boehm, S. Schilling, S. Rosinger et al., “Elevated serum levels of N'-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema,” *Diabetologia*, vol. 47, no. 8, pp. 1376–1379, 2004.

[35] A. A. Ghanem, A. Elewa, and L. F. Arafa, “Pentosidine and N-carboxymethyl-lysine: biomarkers for type 2 diabetic retinopathy,” *European Journal of Ophthalmology*, vol. 21, no. 1, pp. 48–54, 2011.

[36] K. Hirata and K. Kubo, “Relationship between blood levels of N-carboxymethyl-lysine and pentosidine and the severity of microangiopathy in type 2 diabetes,” *Endocrine Journal*, vol. 51, no. 6, pp. 537–544, 2004.

[37] N. Grossin, M.-P. Wautier, T. Meas, P.-J. Guillausseau, P. Massin, and J.-L. Wautier, “Severity of diabetic microvascular complications is associated with a low soluble RAGE level,” *Diabetes and Metabolism*, vol. 34, no. 4, pp. 392–395, 2008.

[38] S. Choudhuri, D. Dutta, A. Sen et al., “Role of N-epsilon-carboxy methyl lysine, advanced glycation end products and reactive oxygen species for the development of nonproliferative and proliferative retinopathy in type 2 diabetes mellitus,” *Molecular Vision*, vol. 19, pp. 100–113, 2013.

[39] M. Kerkeni, A. Saidi, H. Bouzidi, A. Letaief, S. Ben Yahia, and M. Hammami, “Pentosidine as a biomarker for microvascular complications in type 2 diabetic patients,” *Diabetes and Vascular Disease Research*, vol. 10, no. 3, pp. 239–245, 2013.

[40] M. T. Schram, N. Chaturvedi, C. G. Schalkwijk, J. H. Fuller, and C. D. A. Stehouwer, “Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes—the EURODIAB Prospective Complications Study,” *Diabetologia*, vol. 48, no. 2, pp. 370–378, 2005.

[41] A. G. Salman, D. E. A. A. Mansour, A.-H. A. Swelem, W. M. A.-R. Al-Zawahary, and A. A. Radwan, “Pentosidine—a new biochemical marker in diabetic retinopathy,” *Ophthalmic Research*, vol. 42, no. 2, pp. 96–98, 2009.
[42] S. Genuth, W. Sun, P. Cleary et al., "Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes," *Diabetes*, vol. 54, no. 11, pp. 3103–3111, 2005.

[43] M. Sternberg, J. M'Bemma, P. Urios et al., "Skin collagen pentosidine and fluorescence in diabetes were predictors of retinopathy progression and creatininemia increase already 6 years after punch-biopsy," *Clinical Biochemistry*, vol. 49, no. 3, pp. 223–231, 2016.

[44] T. Shinoda, F. Hayase, and H. Kato, "Suppression of cell-cycle progression during the S phase of rat fibroblasts by 3-deoxyglucosone, a Maillard reaction intermediate," *Bioscience, Biotechnology and Biochemistry*, vol. 58, no. 12, pp. 2215–2219, 1994.

[45] A. Okado, Y. Kawasaki, Y. Hasuike et al., "Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines," *Biochemical and Biophysical Research Communications*, vol. 225, no. 1, pp. 219–224, 1996.

[46] H. Kusunoki, S. Miyata, T. Ohara et al., "Relation between methylglyoxal and 3-deoxyglucosone ratio: a potential risk factor determinant for type 2 diabetes," *Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-years after punch-biopsy*, *Diabetes*, vol. 55, no. 1, pp. 86–92, 2006.

[47] S. Genuth, W. Sun, P. Cleary et al., "Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-years after punch-biopsy," *Clinical Biochemistry*, vol. 49, no. 3, pp. 223–231, 2016.

[48] T. Watanabe, K. Negishi, S. Katayama, J. Ishii, and S. Kawazu, "Serum and urinary type IV collagen and fibronectin concentrations in diabetic patients with microangiopathy," *Journal of Korean Medical Science*, vol. 9, no. 4, pp. 341–346, 1994.

[49] R. Mancino, D. DiPierro, C. Varesi et al., "Lipid peroxidation products in diabetes mellitus and diabetic microangiopathy," *British Journal of Ophthalmology*, vol. 92, no. 1, pp. 136–140, 2008.

[50] J. R. Williamson, R. G. Tilton, K. Chang, and C. Kilo, "Basement membrane abnormalities in diabetes mellitus: relationship to clinical microangiopathy," *Diabetes/Metabolism Reviews*, vol. 4, no. 4, pp. 339–370, 1988.

[51] B. M. Chavers, S. M. Mauer, R. C. Ramsay, and M. W. Steffes, "Relationship between retinal and glomerular lesions in IDDM patients," *Diabetes*, vol. 43, no. 3, pp. 441–446, 1994.

[52] T. Bek and T. Ledet, "Glycoprotein deposition in vascular walls of diabetic retinopathy: A histopathological and immunohistochemical study," *Acta Ophthalmologica Scandinavica*, vol. 74, no. 4, pp. 385–390, 1996.

[53] S. Cherian, S. Roy, A. Pinheiro, and S. Roy, "Tight glycemic control regulates fibronectin expression and basement membrane thickening in retinal and glomerular capillaries of diabetic rats," *Investigative Ophthalmology and Visual Science*, vol. 50, no. 2, pp. 943–949, 2009.

[54] P. Polewski, M. Chadda, A.-F. Li, T. Sato, and S. Roy, "Effect of combined antisense oligonucleotides against high-glucose- and diabetes-induced overexpression of extracellular matrix components and increased vascular permeability," *Diabetes*, vol. 55, no. 1, pp. 86–92, 2006.

[55] S. Roy and M. Lorenzi, "Early biosynthetic changes in the diabetic-like retinopathy of galactose-fed rats," *Diabetologia*, vol. 39, no. 6, pp. 735–738, 1996.

[56] I. K. Lee, K. Y. Park, H. K. Oh, R. W. Park, and J. S. Jo, "Plasma type IV collagen and fibronectin concentrations in diabetic patients with microangiopathy," *Journal of Korean Medical Science*, vol. 9, no. 4, pp. 341–346, 1994.

[57] Y. Hayashi, H. Makino, and Z. Ota, "Serum and urinary concentrations of type IV collagen and laminin as a marker of microangiopathy in diabetes," *Diabetic Medicine*, vol. 9, no. 4, pp. 366–370, 1992.

[58] N. Banu, H. Harag, E. Gura, and M. Yamakido, "Serum and urinary type IV collagen concentrations in the assessment of diabetic microangiopathy," *Hiroshima Journal of Medical Sciences*, vol. 43, no. 4, pp. 123–133, 1994.

[59] T. Watanabe, K. Negishi, S. Katayama, J. Ishii, and S. Kawazu, "Serum and urinary concentration of type IV collagen in diabetic patients with diabetic microangiopathy," *Journal of Clinical Laboratory Analysis*, vol. 20, no. 2, pp. 191–192, 1996.

[60] N. Banu, H. Harag, E. Gura, and M. Yamakido, "Serum and urinary type IV collagen concentrations in the assessment of diabetic microangiopathy," *Hiroshima Journal of Medical Sciences*, vol. 43, no. 4, pp. 123–133, 1994.

[61] T. Watanabe, K. Negishi, S. Katayama, J. Ishii, and S. Kawazu, "Serum and urinary concentration of type IV collagen in diabetic patients with diabetic microangiopathy," *Journal of Clinical Laboratory Analysis*, vol. 20, no. 2, pp. 191–192, 1996.

[62] M. Yamakido, M. Suzuki, K. Jinde et al., "Significance of urinary type IV collagen in patients with diabetic nephropathy using a highly sensitive one-step sandwich enzyme immunoassay," *Journal of Clinical Laboratory Analysis*, vol. 11, no. 2, pp. 100–116, 1997.

[63] G. Nicoloff, S. Baydanoff, N. Stamimirova, C. Petrova, and P. Christova, "Detection of serum collagen type IV in children with type 1 (insulin-dependent) diabetes mellitus—a longitudinal study," *Pediatric Diabetes*, vol. 2, no. 4, pp. 184–190, 2001.

[64] N. Kotajima, T. Kanda, N. Yuuki et al., "Type IV collagen serum and vitreous fluid levels in patients with diabetic retinopathy," *Journal of International Medical Research*, vol. 29, no. 4, pp. 292–296, 2001.

[65] E. Caglieri, T. Roth, S. Roy, and A. M. Lorenzi, "Characteristics and mechanisms of high-glucose-induced overexpression of basement membrane components in cultured human endothelial cells," *Diabetes*, vol. 40, no. 1, pp. 102–110, 1991.

[66] G. Pugliese, F. Pricci, F. Pugliese et al., "Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells," *Diabetes*, vol. 43, no. 3, pp. 478–490, 1994.

[67] P. Pietzschmann, G. Scherthanher, C. H. Schneck, and S. Gaube, "Serum concentrations of laminin PI in diabetics with advanced nephropathy," *Journal of Clinical Pathology*, vol. 41, no. 9, pp. 929–932, 1988.

[68] S. Tomono, S. Kawazu, N. Kato, T. Ohno, T. Utsugi, and K. Murata, "Clinical implications of serum levels of basement membrane components in diabetic patients with and without albuminuria," *Journal of Diabetic Complications*, vol. 5, no. 2-3, pp. 193–194, 1991.

[69] E. Werle, E. Diehl, and C. Hasslacher, "Levels and molecular size distribution of serum laminin in adult type I diabetic patients with and without microangiopathy," *Metabolism*, vol. 47, no. 1, pp. 63–69, 1998.

[70] R. Okazaki, K. Matsuoka, A. Horiiuchi, K. Maruyama, and I. Okazaki, "Assays of serum laminin and type III procollagen...
peptide for monitoring the clinical course of diabetic microangiopathy," *Diabetes Research and Clinical Practice*, vol. 5, no. 3, pp. 163–170, 1988.

[72] R. Okazaki, K. Matsuoka, Y. Atsumi, K. Maruyama, H. Matsuki, and I. Okazaki, "Serum concentrations of basement membrane proteins in NIDDM as a prognostic marker for nephropathy," *Diabetes Research and Clinical Practice*, vol. 27, no. 1, pp. 39–49, 1995.

[73] R. Simó, L. Masmiquel, L. García-Pascual et al., "Serum concentrations of laminin-P1 in diabetes mellitus: usefulness as an index of diabetic microangiopathy," *Diabetes Research and Clinical Practice*, vol. 32, no. 1-2, pp. 45–53, 1996.

[74] L. I. Masmiquel, R. Burgos, C. Mateo, R. Martí, R. M. Segura, and R. Simó, "Effect of panretinal photocoagulation on serum levels of laminin in patients with diabetes: a prospective study," *British Journal of Ophthalmology*, vol. 83, no. 9, pp. 1056–1059, 1999.

[75] L. Masmiquel, R. M. Segura, C. Mateo et al., "Serum laminin as a marker of diabetic retinopathy development: a 4-year follow-up study," *American Journal of Ophthalmology*, vol. 129, no. 3, pp. 347–352, 2000.

[76] G. Mohammad and M. M. Siddiquei, "Role of matrix metalloproteinase-2 and -9 in the development of diabetic retinopathy," *Journal of Ocular Biology, Diseases, and Informatics*, vol. 5, no. 1, pp. 1–8, 2012.

[77] R. A. Kowluru, Q. Zhong, and J. M. Santos, "Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9," *Expert Opinion on Investigational Drugs*, vol. 21, no. 6, pp. 797–805, 2012.

[78] S. Jacqueminet, O. Ben Abdesselam, M.-I. Chapman et al., "Elevated circulating levels of matrix metalloproteinase-9 in type 1 diabetic patients with and without retinopathy," *Clinica Chimica Acta*, vol. 367, no. 1-2, pp. 103–107, 2006.

[79] M. Béraneck, P. Kolar, S. Tschoplova, K. Kankova, and A. Vasku, "Genetic variations and plasma levels of gelatinase A (matrix metalloproteinase-2) and gelatinase B (matrix metalloproteinase-9) in proliferative diabetic retinopathy," *Molecular Vision*, vol. 14, pp. 1114–1121, 2008.

[80] P. R. Maxwell, P. M. Timms, S. Chandran, and D. Gordon, "Peripheral blood level alterations of TIMP-1, MMP-2, and MMP-9 in patients with type 1 diabetes," *Diabetic Medicine*, vol. 18, no. 10, pp. 777–780, 2001.

[81] S. A. Peeters, L. Engelen, J. Buijs et al., "Plasma levels of matrix metalloproteinase-2, -3, -10, and tissue inhibitor of metalloproteinase-1 are associated with vascular complications in patients with type 1 diabetes: the EURODIAB Prospective Complications Study," *Cardiovascular Diabetology*, vol. 14, article 31, 2015.

[82] T. S. Kern, "Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy," *Experimental Diabetes Research*, vol. 2007, Article ID 95103, 2007.

[83] J. Tang and T. S. Kern, "Inflammation in diabetic retinopathy," *Progress in Retinal and Eye Research*, vol. 30, no. 5, pp. 343–358, 2011.

[84] R. Simó and C. Hernández, "Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence," *Progress in Retinal and Eye Research*, vol. 48, pp. 160–180, 2015.

[85] O. Simó-Servat, C. Hernández, and R. Simó, "Usefulness of the vitreous fluid analysis in the translational research of diabetic retinopathy," *Mediators of inflammation*, vol. 2012, Article ID 872978, 11 pages, 2012.

[86] A. M. A. El-Asrar, "Role of inflammation in the pathogenesis of diabetic retinopathy," *Middle East African Journal of Ophthalmology*, vol. 19, no. 1, pp. 70–74, 2012.
[128] Q. Yang, T. E. Graham, N. Mody et al., “Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes,” *Nature*, vol. 436, no. 7049, pp. 356–362, 2005.

[129] P. Balagopal, T. E. Graham, B. B. Kahn, A. Altomare, V. Funanage, and D. George, “Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation,” *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 5, pp. 1971–1974, 2007.

[130] K. M. Farjo, R. A. Farjo, S. Halsey, G. Moiseyev, and J.-X. Ma, P. Balagopal, T. E. Graham, B. B. Kahn, A. Altomare, V. Funanage, and D. George, “Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation,” *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 5, pp. 1971–1974, 2007.

[131] K. Takebayashi, M. Suetsugu, S. Wakabayashi, Y. Aso, and T. Imakai, “Retinol-binding protein 4 levels and clinical features of type 2 diabetes patients,” *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 7, pp. 2712–2719, 2007.

[132] Z.-Z. Li, X.-Z. Lu, J.-B. Liu, and L. Chen, “Serum retinol-binding protein 4 levels in patients with diabetic retinopathy,” *Journal of International Medical Research*, vol. 38, no. 1, pp. 95–99, 2010.

[133] A. Cabrè, I. Lázaro, J. Girona et al., “Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes,” *Journal of Internal Medicine*, vol. 262, no. 4, pp. 496–503, 2007.

[134] E. Yıldız, N. Muslu, E. Nayir, O. Ozhan, and A. Kiykim, “Serum retinol binding protein 4 level is related with renal functions in Type 2 diabetes,” *Journal of Endocrinological Investigation*, vol. 33, no. 10, pp. 725–729, 2010.

[135] K. Kitamura, K. Kagawa, M. Kawamoto et al., “Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma,” *Biochemical and Biophysical Research Communications*, vol. 192, no. 2, pp. 553–560, 1993.

[136] J. Sakata, T. Shimokubo, K. Kitamura et al., “Distribution and characterization of immunoreactive rat adrenomedullin in tissue and plasma,” *FEBS Letters*, vol. 352, no. 2, pp. 105–108, 1994.

[137] S. Sugio, N. Minamino, K. Kagawa et al., “Endothelial cells actively synthesize and secrete adrenomedullin,” *Biochemical and Biophysical Research Communications*, vol. 201, no. 3, pp. 1160–1166, 1994.

[138] J. P. Hinson, S. Kapas, and D. M. Smith, “Adrenomedullin, a multifunctional regulatory peptide,” *Endocrine Reviews*, vol. 21, no. 2, pp. 138–167, 2000.

[139] R. Udono-Fujimori, T. Udono, K. Totsune, M. Tamai, S. Shibahara, and K. Takahashi, “Adrenomedullin in the eye,” *Regulatory Peptides*, vol. 112, no. 1–3, pp. 95–101, 2003.

[140] M. Hayashi, T. Shimosawa, M.-A. Isaka, S. Yamada, R. Fujita, and T. Fujita, “Plasma adrenomedullin in diabetes,” *The Lancet*, vol. 350, no. 9089, pp. 1449–1450, 1997.

[141] S. Ito, K. Fujisawa, T. Sakamoto, and T. Ishibashi, “Elevated adrenomedullin in the vitreous of patients with diabetic retinopathy,” *Ophthalmologica*, vol. 217, no. 1, pp. 53–57, 2003.

[142] Y. Lu, Y. Xu, and C. Tang, “Changes in adrenomedullin in patients with proliferative diabetic retinopathy,” *Current Eye Research*, vol. 36, no. 11, pp. 1047–1052, 2011.

[143] C. Caliumi, S. Balducci, L. Petramala et al., “Plasma levels of adrenomedullin, a vasoactive peptide, in type 2 diabetic patients with and without retinopathy,” *Minerva Endocrinologica*, vol. 32, no. 2, pp. 73–78, 2007.

[144] T. Nakamura, K. Honda, S.-E. Ishikawa, K. Kitamura, T. Eto, and T. Saito, “Plasma adrenomedullin levels in patients with non-insulin-dependent diabetes mellitus: close relationships with diabetic complications,” *Endocrine Journal*, vol. 45, no. 2, pp. 241–246, 1998.

[145] K. Semeran, P. Pawłowski, L. Lisowski et al., “Plasma levels of il-17, VEGF, and adrenomedullin and s-cone dysfunction of the retina in children and adolescents without signs of retinopathy and with varied duration of diabetes,” *Mediators of Inflammation*, vol. 2013, Article ID 274726, 8 pages, 2013.

[146] G. N. Welch and J. Loscalzo, “Homocysteine and atherosclerosis,” *The New England Journal of Medicine*, vol. 338, no. 15, pp. 1042–1043, 1998.

[147] H. C. Cho, “The relationship among homocysteine, bilirubin, and diabetic retinopathy,” *Diabetes and Metabolism Journal*, vol. 35, no. 6, pp. 595–601, 2011.

[148] C. P. Lim, A. V. P. Loo, K. W. Khaw et al., “Plasma, aqueous and vitreous homocysteine levels in proliferative diabetic retinopathy,” *British Journal of Ophthalmology*, vol. 96, no. 5, pp. 704–707, 2012.

[149] M. V. Van Hecke, J. M. Dekker, G. Nijpels et al., “Homocysteine, S-adenosylmethionine and S-adenosylhomocysteine are associated with retinal microvascular abnormalities: the Hoorn Study,” *Clinical Science*, vol. 114, no. 7–8, pp. 479–487, 2008.

[150] M. Goldstein, I. Leibovitch, I. Yeffimov, S. Gavendo, B.-A. Sela, and A. Loewenstein, “Hyperhomocysteinemia in patients with diabetes mellitus with and without diabetic retinopathy,” *Eye*, vol. 18, no. 5, pp. 460–465, 2004.

[151] O. Aydemir, P. Türkcüoğlu, M. Güler et al., “Plasma and vitreous homocysteine concentrations in patients with proliferative diabetic retinopathy,” *Retina*, vol. 28, no. 5, pp. 741–743, 2008.

[152] H. C. Looker, A. Fagot-Campagna, E. W. Gunter et al., “Homocysteine as a risk factor for nephropathy and retinopathy in type 2 diabetes,” *Diabetologia*, vol. 46, no. 6, pp. 766–772, 2003.

[153] I. Yücel, G. Yücel, and F. Müftüoğlu, “Plasma homocysteine levels in noninsulin-dependent diabetes mellitus with retinopathy and neovascular glaucoma,” *International Ophthalmology*, vol. 25, no. 4, pp. 201–205, 2004.

[154] G. Malaguarnera, C. Gagliano, S. Salomone et al., “Folate status in type 2 diabetic patients with and without retinopathy,” *Clinical Ophthalmology*, vol. 9, pp. 1437–1442, 2015.

[155] B. E. K. Klein, M. D. Knudtson, M. Y. Tsai, and R. Klein, “The relation of markers of inflammation and endothelial dysfunction to the prevalence and progression of diabetic retinopathy: Wisconsin epidemiologic study of diabetic retinopathy,” *Archives of Ophthalmology*, vol. 127, no. 9, pp. 1175–1182, 2009.

[156] E. Aydin, H. D. Demir, H. Ozuyurt, and I. Etikan, “Association of plasma homocysteine and macular edema in type 2 diabetes mellitus,” *European Journal of Ophthalmology*, vol. 18, no. 2, pp. 226–232, 2008.

[157] D. A. de Luis, N. Fernandez, M. L. Arranz, R. Aller, O. Izoa, and E. Romero, “Total homocysteine levels relation with chronic complications of diabetes, body composition, and other cardiovascular risk factors in a population of patients with diabetes mellitus type 2,” *Journal of Diabetes and its Complications*, vol. 19, no. 1, pp. 42–46, 2005.

[158] C.-D. Agardh, E. Agardh, A. Andersson, and B. Hultberg, “Lack of association between plasma homocysteine levels and...
microangiopathy in type 1 diabetes mellitus,” *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 54, no. 8, pp. 637–641, 1994.

[160] L. Brazionis, K. Rowley Sr., C. Itsiopoulos, C. A. Harper, and K. O’Dea, “Homocysteine and diabetic retinopathy,” *Diabetes Care*, vol. 31, no. 1, pp. 50–56, 2008.

[161] M. Sugai, A. Ohta, Y. Ogata et al., “Asymmetric dimethylarginine (ADMA) in the aqueous humor of diabetic patients,” *Endocrine Journal*, vol. 54, no. 2, pp. 303–309, 2007.

[162] S. Abhary, N. Kasmeridis, K. P. Burdon et al., “Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines,” *Diabetes Care*, vol. 32, no. 11, pp. 2084–2086, 2009.

[163] M. T. Malecki, A. Undas, K. Cyganek et al., “Plasma asymmetric dimethylarginine (ADMA) is associated with retinopathy in type 2 diabetes,” *Diabetes Care*, vol. 30, no. 11, pp. 2899–2901, 2007.

[164] P. Vallence, A. Leone, A. Calver, J. Collier, and S. Moncada, “Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis,” *Journal of Cardiovascular Pharmacology*, vol. 20, no. 12, pp. S60–S62, 1992.

[165] R. H. Boger, S. M. Bode-Boger, A. Szuba et al., “Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia,” *Circulation*, vol. 98, no. 18, pp. 1842–1847, 1998.

[166] O. Suda, M. Tsutui, N. Kasmeridis, K. P. Burdon et al., “Asymmetric dimethylarginine (ADMA) in the aqueous humor of diabetic patients,” *Endocrine Journal*, vol. 54, no. 2, pp. 303–309, 2007.

[167] S. Abhary, N. Kasmeridis, K. P. Burdon et al., “Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines,” *Diabetes Care*, vol. 32, no. 11, pp. 2084–2086, 2009.

[168] M. Sugai, A. Ohta, Y. Ogata et al., “Asymmetric dimethylarginine (ADMA) in the aqueous humor of diabetic patients,” *Endocrine Journal*, vol. 54, no. 2, pp. 303–309, 2007.

[169] S. Abhary, N. Kasmeridis, K. P. Burdon et al., “Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines,” *Diabetes Care*, vol. 32, no. 11, pp. 2084–2086, 2009.

[170] M. T. Malecki, A. Undas, K. Cyganek et al., “Plasma asymmetric dimethylarginine (ADMA) is associated with retinopathy in type 2 diabetes,” *Diabetes Care*, vol. 30, no. 11, pp. 2899–2901, 2007.

[171] P. Vallence, A. Leone, A. Calver, J. Collier, and S. Moncada, “Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis,” *Journal of Cardiovascular Pharmacology*, vol. 20, no. 12, pp. S60–S62, 1992.

[172] R. H. Boger, S. M. Bode-Boger, A. Szuba et al., “Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia,” *Circulation*, vol. 98, no. 18, pp. 1842–1847, 1998.

[173] O. Suda, M. Tsutui, N. Kasmeridis, K. P. Burdon et al., “Asymmetric dimethylarginine (ADMA) in the aqueous humor of diabetic patients,” *Endocrine Journal*, vol. 54, no. 2, pp. 303–309, 2007.

[174] S. Abhary, N. Kasmeridis, K. P. Burdon et al., “Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines,” *Diabetes Care*, vol. 32, no. 11, pp. 2084–2086, 2009.

[175] G. P. Fadini, M. Miorini, M. Facco et al., “Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus,” *Journal of the American College of Cardiology*, vol. 45, no. 9, pp. 1449–1457, 2005.
[190] K. Hamaoui, A. Butt, J. Powrie, and R. Swaminathan, “Concentration of circulating rhodopsin mRNA in diabetic retinopathy,” Clinical Chemistry, vol. 50, no. 11, pp. 2152–2155, 2004.

[191] P. A. Hargrave, “Rhodopsin structure, function, and topology: the Friedenwald lecture,” Investigative Ophthalmology and Visual Science, vol. 42, no. 1, pp. 3–9, 2001.

[192] A. Butt, M. S. Ahmad, J. Powrie, and R. Swaminathan, “Assessment of diabetic retinopathy by measuring retina-specific mRNA in blood,” Expert Opinion on Biological Therapy, vol. 12, supplement 1, pp. S79–S84, 2012.

[193] Z. Shalchi, H. S. Sandhu, A. N. Butt, S. Smith, J. Powrie, and R. Swaminathan, “Retina-specific mRNA in the assessment of diabetic retinopathy,” Annals of the New York Academy of Sciences, vol. 1137, pp. 253–257, 2008.

[194] V. Ambros, “The functions of animal microRNAs,” Nature, vol. 431, no. 7006, pp. 350–355, 2004.

[195] D. P. Bartel, “MicroRNAs: genomics, biogenesis, mechanism, and function,” Cell, vol. 116, no. 2, pp. 281–297, 2004.

[196] D. P. Bartel, “MicroRNAs: target recognition and regulatory functions,” Cell, vol. 136, no. 2, pp. 215–233, 2009.

[197] M. Kato, N. E. Castro, and R. Natarajan, “MicroRNAs: potential mediators and biomarkers of diabetic complications,” Free Radical Biology and Medicine, vol. 64, pp. 85–94, 2013.

[198] R. Natarajan, S. Putta, and M. Kato, “MicroRNAs and diabetic complications,” Journal of Cardiovascular Translational Research, vol. 5, no. 4, pp. 413–422, 2012.

[199] A. D. Mcclelland and P. Kantharidis, “MicroRNA in the development of diabetic complications,” Clinical Science, vol. 126, no. 2, pp. 95–110, 2014.

[200] B. Feng, S. Chen, K. McArthur et al., “miR-146a-mediated extracellular matrix protein production in chronic diabetes complications,” Diabetes, vol. 60, no. 11, pp. 2975–2984, 2011.

[201] K. McArthur, B. Feng, Y. Wu, S. Chen, and S. Chakrabarti, “MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy,” Diabetes, vol. 60, no. 4, pp. 1314–1323, 2011.

[202] V. A. Silva, A. Polesskaya, T. A. Sousa et al., “Expression and cellular localization of microRNA-29b and RAX, an activator of the RNA-dependent protein kinase (PKR), in the retina of streptozotocin-induced diabetic rats,” Molecular Vision, vol. 17, pp. 2228–2240, 2011.

[203] N. García de la Torre, R. Fernández-Durango, R. Gómez et al., “Expression of angiogenic MicroRNAs in endothelial progenitor cells from type 1 diabetic patients with and without diabetic retinopathy,” Investigative Ophthalmology & Visual Science, vol. 56, no. 6, pp. 4090–4098, 2015.