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Tear-fluid-derived biomarkers of ocular complications in diabetes: a systematic review and meta-analysis

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Abstract

Background Early identification and management of sight-threatening ocular complications of diabetes using imaging or molecular biomarkers could help prevent vision loss. However, access to specialized infrastructure and expertise is limited, especially in remote areas of the world. Tear-fluid may offer an easier, non-invasive, and localized screenshot of ocular disease. To the best of our knowledge, there is no systematic review and meta-analysis on tear-fluid-based biomarkers for ocular complications in diabetes.

Methods Articles were extracted from PubMed, Embase, Medline, and Web of Science using the MeSH and Emtree terms. The keywords include (diabetes), (diabetic retinopathy), (diabetes mellitus, type 1), (diabetes mellitus, type 2), (insulin-dependent diabetes), (insulin resistant diabetes), (tears), (lacrimal fluid), (biological marker), and (biomarker, marker). Concentrations of tear-fluid biomarkers in individuals with diabetes, diabetic ocular complications, and healthy controls were extracted and standardized mean differences (SMDs) and 95% CIs were calculated. Heterogeneity was assessed using subgroup and leave-one-out sensitivity analyses. Publication and risk of bias were performed using the Egger's test and Cochrane guidelines. The quality of evidence was evaluated using the Newcastle–Ottawa scale.

Results Nine hundred eleven papers were identified, 19 of which met the study criteria and were included in the meta-analysis. Participants ($n=1413$) belonged to three groups: healthy controls (Controls), diabetes without any complications (Diabetes), and diabetes with ocular complications (Complications). Actual concentrations were reported for TNF-α, VEGF, IL-1RA, IL-1β, IL-6, IL-8, lactoferrin, lysozyme, and MCP-1 in at least three different studies. Meta-analyses demonstrated that TNF-α concentration was significantly higher in the tear-fluid of Complications group when compared to Controls (SMD = −1.08, 95% CIs = −1.78, −0.38, $p=0.003$) or when compared to Diabetes (SMD = −0.78, 95% CIs = −1.48, −0.09, $p=0.03$). However, it was not different when Controls were compared to Diabetes (SMD = −1.00, 95% CIs = −2.27, 0.28, $p=0.13$). VEGF demonstrated a similar trend indicating specificity of tear-fluid TNF-α and VEGF for diabetic ocular complications.

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Conclusions Across all biomolecules meta-analyzed in this study, TNF-α and VEGF were identified as the most important biomarkers that could potentially offer a non-invasive tear-fluid-based assessment of progression to ocular complications in diabetes, especially in rural and remote areas where diabetes-related expertise and infrastructure are limited.

Trial registration PROSPERO (CRD42023441867)

https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=441867.

Keywords Diabetes, Islet, Diabetic ocular complications, Biomarker discovery, Tears, Tear-fluid, Cytokines, Proteins, Risk stratification

Background

Diabetes mellitus (DM) is a progressive, complex metabolic disorder that affects 529 million individuals globally [1]. DM is characterized by hyperglycaemia that results from dead/dysfunctional beta cells or insulin resistance [2]. Prolonged exposure to high glucose conditions often leads to microvascular complications, damaging different organs, including the eyes [3]. Ocular diabetic complications such as diabetic retinopathy (DR) are currently one of the leading causes of blindness. Global prevalence of DR is high and is estimated to increase to up to 130 million individuals in 2030 [4]. Other diabetic ocular complications include diabetic corneal neuropathy (DCN), DM with dry eye disease (DED), and diabetic macular edema, among others [5].

Currently, there are a variety of screening methods that are used to diagnose ocular complications including imaging techniques and analysis of biomarkers from biofluids (serum, plasma, urine, saliva, aqueous and vitreous humor, and tears) [6, 7]. Imaging techniques such as optical coherence tomography (OCT) and retinal fundus photographs are the gold standard for identifying diabetic ocular complications; however, these diagnostic methods require high-quality ophthalmic imaging instrumentation that are not only difficult to obtain in rural and remote areas, but these imaging techniques also demand specialized infrastructure and ophthalmologists for grading the images [8–11]. Tear-fluid offers a high potential for biomarker assessment for the diagnosis of ocular complications, as these samples can be accessed with ease through non-invasive methods such as collection via microcapillary tubes, Schirmer's strips, micropipette tips or sponges [12–14]. Currently, new diagnostic tools are being developed to assess tear-fluid-based biomolecules using hands-on or point-of-care devices similar to a COVID rapid antigen test kit. Additionally, tear-fluid samples with their close proximity to the affected organ offer a more localized complement of biomarkers such as cytokines, proteins, and microRNAs (miRNAs) [12, 15, 16] that may prove useful in detecting ocular complications.

There is currently no systematic review and meta-analysis that is focussed on tear-fluid-based biomarkers of ocular disease in individuals with diabetes. Existing analyses in tear-fluids have either been systematic reviews [17–20] or meta-analyses that are focussed on dry eye disease (DED [21]), keratoconus [22], or other ocular conditions with focus on a specific biomolecule: lactoferrin [23]. These are tabled under Additional File 1: Table S1 [17–23]. It is therefore essential to undertake a systematic review and meta-analysis of all available tear-fluid-based biomarkers of diabetic ocular complications.

We aimed to systematically analyze the results of all case–control and observational studies that reported the concentration of various biomarkers within tear-fluid from individuals across the following groups: (1) healthy controls, (2) those with diabetes but no complications, and (3) those with diabetes and associated ocular complications.

Methods

This systematic review and meta-analysis was conducted and reported under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guideline and checklist [24]. Details regarding search strategy, eligibility criteria, data extraction, and analysis of extracted data are outlined in the PROSPERO registration (ID no. CRD42023441867).

Literature search

PRISMA guidelines were used to systematically search PubMed, Embase, Medline, and Web of Science databases and extract data from human studies that measured tear-fluid biomarkers in healthy controls or individuals with diabetes, with or without ocular complications. Articles published between the creation of each database and October 21, 2024, were screened. The article search was not language restricted. We searched each database using the defined keywords and their synonyms in the MeSH (Medical Subject Heading) and Emtree terms. The keywords include (diabetes), (diabetic retinopathy), (diabetes mellitus, type 1), (diabetes mellitus, type 2), (insulin dependent diabetes), (insulin resistant diabetes), (tears), (lacrimal fluid), (biological marker), and (biomarker, marker). The details of search strategies are provided in

Additional File 1: Table S2. All papers were screened for titles, abstracts, and full-text (Fig. 1).

Inclusion and exclusion criteria

All records from each search were imported into Microsoft Excel. Duplicate articles were removed, and the remaining articles were manually selected following the screening of the title and abstract. Articles were only included if they met the PICOS (Participants, Intervention, Comparators, Outcome, Study design) criteria that are outlined in Table 1. Following inclusion criteria were applied: (i) human studies, (ii) original articles only, (iii) full text available, (iv) reporting tear-fluid-based biomarkers with actual concentrations (in table or figures) in at least three different studies, and (v) case–control and observational studies with healthy controls and individuals with diabetes, and without or with ocular complications.

Data extraction

Demographic information of participants and biomarker concentrations from the final selected studies were

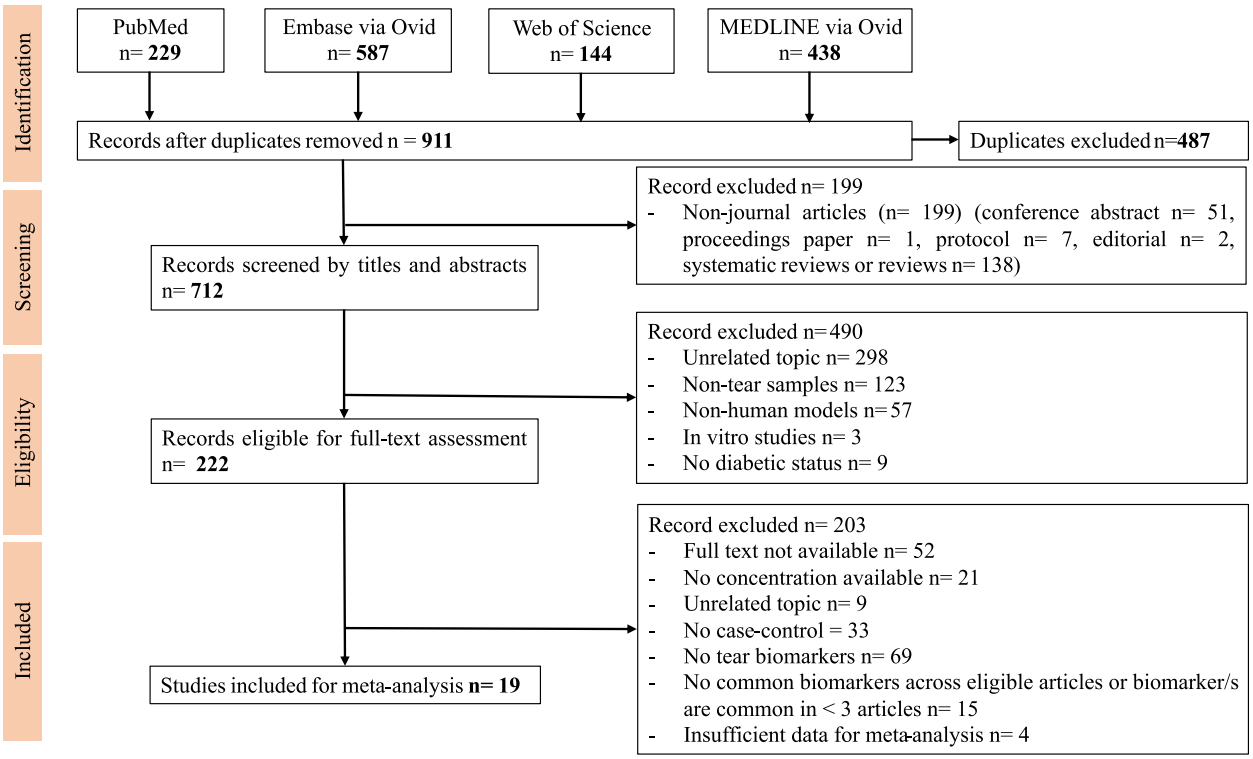

Fig. 1 PRISMA flow diagram outlining the selection process that was undertaken for the systematic review and meta-analysis

Table 1 PICOS criteria for inclusion and exclusion of studies

Parameter	Inclusion criteria	Exclusion criteria
Participants	Healthy individuals; individuals with type 1 or type 2 diabetes without ocular complications; individuals with diabetes and ocular complications	Individuals with ocular complications in the absence of diabetes
Interventions	No interventions or treatment before sample collection	Treatment or any drug intervention at the time of sample collection
Comparators	Controls versus Complications; Controls versus Diabetes; Diabetes versus Complications	Longitudinal time points (baseline vs endpoint)
Outcomes	Biomarkers with actual concentrations reported in tear-fluid	Studies not reporting biomarkers and/or concentrations for the reported biomarker
Study design	Case–control and observational studies	Non-original articles (conference abstracts, proceedings papers, reviews, systematic reviews, protocols, meta-analyses), drug interventions

extracted into a Microsoft Excel worksheet. All values were converted to mean ± standard deviation (SD) for the concentrations of biomarkers that were presented as mean + standard error of mean (SEM), median + interquartile range (IQR) or min-max [25]. In one of the studies [26], units or SD were confirmed via author correspondence. For 2 of the 19 studies [27, 28], which presented the data as figures, values were extracted from the figures using the free online platform WebPlotDigitizer, version 4.8 [29]; after which, mean ± SD were calculated in Excel. Although this method [29] is validated for data extraction, we confirmed in our hands that the method can be reliably used on different types of figures (e.g., bar plots, scatter plots, line graphs). Every step of the meta-analysis (database search, screening, and data extraction) was performed by a minimum of two researchers independently. A very high (>95%) degree of agreement was observed between the independent search strategy among the researchers. In case of any disagreement, WKMW and MVJ resolved the conflicts followed by the team consensus.

Quality assessment

Newcastle–Ottawa Scale (NOS) [30] was used for quality assessment of the studies included in the meta-analysis. Additional parameters for data and method transparency were included in the questionnaire. Scores of ≤6, 7–8, and 9–10 were considered as low-, medium-, and high-quality of evidence, respectively. Additional risk of bias was conducted in accordance with the Cochrane assessment for randomized studies [31] and in accordance with the ROBINS-I tool for observational studies [32], and the outcomes were presented using RevMan version 5.4. Publication bias was assessed using funnel plots and Egger's test [33]. Effect estimate and standard error were used for this analysis using funnel() function in meta package in R [34]. Asymmetry of the funnel plots was estimated using Egger's test of the intercept for funnel plot asymmetry using metabias() function in meta package [34]; and the results were validated using another function eggers.test() from dmetar package in R [35].

Statistical analysis

RevMan 5.4 software was used to generate forest plots for the tear-fluid-based biomarker concentrations that were extracted from the 19 included articles. Subgroup analyses were also performed in RevMan 5.4. All data were entered as mean ± SD. The random-effects analysis model and inverse variance method were selected to evaluate the standardized mean differences (SMD) with 95% confidence intervals (CIs) between the groups. A p-value of ≤0.05 was considered significant. Heterogeneity was presented in each forest plot using different values (Tau^2, Chi^2, I^2). We used I^2 threshold (>70%) to indicate the high level of heterogeneity as per Cochrane guidelines. The leave-one-out sensitivity analysis was performed as described by Harrer et al. [36]. The results were visualized as forest plots using the "Data" element of the "InfluenceAnalysis" R object generated by the "dmetar::InfluenceAnalysis()" function.

Results

Characteristics of studies included in the meta-analysis

Figure 1 illustrates the PRISMA workflow used to select the articles that are included in this meta-analysis. The initial search in PubMed, Embase, Medline, and Web of Science identified 911 articles after excluding 487 duplicates. During title and abstract screening, 689 articles were excluded. The remaining 222 articles were full-text screened, and a final total of 19 articles containing concentrations for commonly reported biomarkers were included in the meta-analysis (Table 2 [26–28, 37–52]). All biomarkers were measured in tear-fluid samples, which were collected via methods such as Schirmer test/strips or glass microcapillaries/pipettes/tubes. All included articles reported concentrations of the selected biomarkers for two or three groups: (healthy controls (Controls), participants with diabetes (Diabetes), and participants with ocular complications of diabetes (Complications)).

Table 2 summarizes the characteristics of the 19 articles selected for meta-analysis, including data from a total of 1413 participants (430 Controls, 273 Diabetes, 710 Complications). During full-text search, we noted several molecules that were measured in the tears (Additional File 1: Table S3); however nine analytes: tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), interleukin-1 receptor agonist (IL-1RA), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), lactoferrin, lysozyme, and monocyte chemoattractant protein-1 (MCP-1) were reported in three or more papers and were included in meta-analysis. The studies were performed in various countries and ethnic groups. Thirteen studies were performed in Asian regions [28, 37, 38, 40–46, 48, 50, 51], and 5 studies were in European regions [26, 27, 39, 47, 49]. One study was conducted in North America [52].

Comparison of biomarker concentrations between participant groups

Concentrations of TNF-α, VEGF, IL-1RA, IL-1β, IL-6, and IL-8 were available for comparison in all three groups, i.e., Controls, Diabetes, and Complications groups.

TNF-α, VEGF, and IL-6 were significantly elevated in the Complications group as opposed to Controls: TNF-α

Table 2 Characteristics of studies included in meta-analysis

Reference; country	Tear sample collection method	Method of quantification	Participants in the Control group			Participants in the Diabetes group			Participants in the Complications group			Reported biomarkers
			Number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	Number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	Complication; number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	
Mei et al [37]; China	Capillary collection strip	Electrochemical platform; ELISA	5	-	-	-	-	-	DR: 5	-	-	VEGF
Sorkhabi et al [41]; Iran	Capillary tube	ELISA	20	8 M and 12 F	46.05±13	20	12 M and 8 F	47.75±5.6	NPDR: 25; PDR: 25	NPDR: 13 M and 12 F; PDR: 10 M and 15 F	NPDR: 52.04±8.7; PDR: 52.92±10.1	TNF-α, IL-8 and IL-1RA
Liu et al [42]; China	Glass capillary tube	ELISA; mulitplex cytokine analysis	15	8 M and 7 F	59±2.42	15	8 M and 7 F	61.27+1.95	DR: 15	7 M and 8 F	61.07±2.16	IL-1β, IL-1RA, IL-6, IL-8, IL-10, VEGF and MCP-1
Azhan et al [43]; Malaysia	Schirmer strip	ELISA	33	19 M and 14 F	69.6±7.5	-	-	-	NPDR: 32	18 M and 14 F	67.3±7.0	VEGF
Ang et al [52]; USA	Schirmer strip	Human immunoassay kit	-	-	-	30	17 M and 13 F	64.6±8.8	NPDR: 28; PDR: 30	NPDR: 11 M and 17 F; PDR: 16 M and 14 F	NPDR: 58.5±8.2; PDR: 52.3±9.6	VEGF
Kim et al [44]; South Korea	Capillary tube	Multiplex immunobead assay; magnetic luminex screening assay	30	15 M and 15 F	67.5 (60–72)	30	7 M and 23 F	70 (63–74)	-	-	-	MCP-1, IL-6, IL-8, and VEGF
Manchikanti et al [38]; India	10 µl micropipette tip attached to a plastic pipette	ELISA	21	19 M and 2 F	51.33±10.683	-	-	-	NPDR: 10; PDR: 11	19 M and 2 F	54.59±11.58	TNF-α and IL-1β
Amorim et al [26]; Portugal	Schirmer I test	BCA protein assay kit; LC-MS/MS; multiplex analysis	12	3 M and 9 F	54±11	13	7 M and 6 F	59±11	NPDR: 25; PDR: 16	NPDR: 18 M and 7 F; PDR: 13 M and 3 F	NPDR: 65±9; PDR: 66±6	IL-1β and IL-6
Byambajav et al [39]; UK	Glass capillary micropipette	Magnetic bead panel	17	6 M and 11 F	52±16	41	25 M and 16 F	62±21	DED: 47	29 M and 18 F	64±16	IL-6, TNF-α, VEGF, IL-1β, IL-1RA, and IL-8
Zhou et al [45]; China	Glass capillary tube	Immune bead–based array	22	10 M and 12 F	57.32±9.66	-	-	-	DR: 48	29 M and 19 F	59.15 ± 9.51	TNF-α and IL-6
Liu et al [40]; China	Glass capillary micropipette	Multiplex bead analysis	29	5 M and 24 F	62.4±7.5	24	7 M and 17 F	63.5±10.1	DED: 32	14 M and 18 F	61.8±9.8	TNF-α and IL-1β
Hashemi et al [46]; Iran	Schirmer's standard strip	ELISA	30	15 M and 15 F	56.90±8	30	16 M and 14 F	58.8±7.74	DR: 30	14 M and 16 F	62.2±7.0	VEGF
Machalińska et al [47]; Poland	Schirmer's standard strip	Multiplex fluorescent bead-based immunoassays, High sensitivity cytokine assay	52	25 M and 27 F	63±12	-	-	-	DR: 52	35 M and 17 F	65±11.5	TNF- α, VEGF, IL-1β, IL-6, and IL-8

Table 2 (continued)

Reference; country	Tear sample collection method	Method of quantification	Participants in the Control group			Participants in the Diabetes group			Participants in the Complications group			Reported biomarkers
			Number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	Number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	Complication; number of participants	Sex	Age (years) Presented as mean ± SD or range or median (IQR)	
Sheikhrezaee et al [28]; Iran	Schirmer's standard strip	ELISA	30	15 M and 15 F	56.9±8	30	16 M and 14 F	58.8±7.8	DR: 30	14 M and 16 F	62.2±7	VEGF
Costagliola et al [27]; Italy	Schirmer's standard strip	ELISA	16	8 M and 8 F	53 (47-58)	-	-	-	NPDR: 16; PDR: 16	NPDR: 9 M and 7 F; PDR: 10M and 6 F	NPDR: 54 (49-67); PDR: 59 (52-73)	TNF-α
Amil-Bangsa et al [48]; Malaysia	Glass capillary tube	ELISA	-	-	-	15	8 M and 7 F	58.3±4.8	Mild NPDR: 15; moderate NPDR: 15; severe NPDR: 15	Mild: 6 M and 9 F, moderate: 9 M and 6 F; severe 12 M and 3 F	Mild: 57.0±4.8; moderate: 58.9±4.7; severe: 57.5±4.8	TNF-α
Stolwijk et al [49]; Netherlands	Glass capillary tube	Bradford's assay, HPLC, SDS-PAGE	26	-	39.7±11.5	25	-	33.4±12.1	DR: 29	-	46.3±10.4	LTF and LZM
Zou et al [50]; China	Schirmer I test	TMT Labeling, HPLC, LC-MS	5	2 M and 3 F	54.80±6.06	-	-	-	DED: 10	4 M and 6 F	55.50±4.79	LTF and LZM
Yu et al [51]; China	Glass micro-capillary tube	Lowry method, SDS-PAGE	67	32 M and 35 F	-	-	-	-	NPDR: 70; PDR: 63	NPDR: 36 M and 34 F; PDR: 31 M and 35 F	-	LTF and LZM
Total participants: 1413			430			273			710			

Characteristics of 19 studies included in the meta-analysis

BCA bicinchoninic acid, *DED* dry eye disease, *DR* diabetic retinopathy, *ELISA* enzyme-linked immunosorbent assay, *EV* extracellular vesicle, *F* female, *HPLC* high-performance liquid chromatography, *IL-1β* interleukin-1 beta, *IL-1RA* interleukin-1 receptor antagonist, *IL-6* interleukin-6, *IL-8* interleukin-8, *LC-MS/MS* liquid chromatography-mass spectrometry/mass spectrometry, *LTF* lactoferrin, *LZM* lysozyme, *M* male, *MCP-1* monocyte chemoattractant protein-1, *NPDR* non-proliferative diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *SDS-PAGE* sodium dodecyl-polyacrylamide gel electrophoresis, *TBUT* tear break up time, *TMT* tandem mass tag, *TNF-α* tumor necrosis factor-alpha, *VEGF* vascular endothelial growth factor

(SMD = −1.08; 95% CI = −1.78, −0.38; $p = 0.003$), VEGF (SMD = −1.44; 95% CI = −2.56, −0.32; $p = 0.01$), and IL-6 (SMD = −0.56; 95% CI = −0.87, −0.24; $p = 0.0006$) (Fig. 2). Other three analytes were not statistically different between these two groups. Interestingly, lactoferrin and lysozyme were higher and overall significant in Controls than in Complications participants (Additional File 1: Figure S1).

IL-6 and IL-8 concentrations were significantly higher in the Diabetes group as compared to Controls, while TNF-α, VEGF, IL-1RA, and IL-1β did not show any statistically significant difference (Fig. 3). MCP-1 expression in tears was similar for these two groups (Additional File 1: Figure S2).

TNF-α, IL-6 and VEGF indicated significantly higher concentrations in the Complications group compared to the Diabetes group (Fig. 4).

Heterogeneity analysis of the included studies

Analyses of the majority of these molecules in different comparisons demonstrated high heterogeneity ($I^2 > 70\%$) for individual as well as for overall analyses (Figs. 2, 3 and 4). Interestingly, we did not find any specific study introducing heterogeneity, with marginal to modest changes observed in the I^2 statistics after leave-one-out sensitivity analysis (Additional File 1: Figure S3-S5). Some articles demonstrated reduction in I^2; however, no single study was observed to introduce heterogeneity across all comparisons and all molecules.

Subgroup analyses were undertaken to understand the contribution of potential factors (methods of tear collection, methods of biomarker analysis, ethnicities, and use of data derivation techniques) to heterogeneity in results. As there were not enough papers after segregating them for data extraction method (WebPlotDigitizer derived graphical data vs directly reported tabular data) or ethnicities or for biomarker measurement method (majority ELISAs), we could not report the subgroup differences and the heterogeneity thereof. The method of capillary-based tear collection was observed to reduce the I^2 values than those with Schirmer paper collection (Additional File 1: Figure S6).

New technologies for biomarker measurement

During the full-text screening, we identified studies that aimed at developing new technologies for the effective measurement of tear-fluid biomarkers. In addition to the meta-analysis of biomarkers (Figs. 2, 3 and 4, Additional File 1: Table S3), our systematic review identified 13 studies that developed and validated methods such as biochips and immuno-sensing platforms for biomarker analysis from tear-fluid samples. A list of these methodologies for the identification of targeted biomarkers of diabetic ocular complications is presented in Table 3 [37, 53–64]. These techniques could be translated for generating hands-on diagnostic sensors in the future.

Quality of evidence assessment

Every study included in the meta-analysis was assessed using Newcastle–Ottawa scale (NOS; Additional File 1: Table S4). In addition to the selection, comparability, and exposure questionnaire, we also analyzed transparency in reporting data and methodological details for each study. The majority of the studies (11 out of 19) had medium to high quality of evidence (scores of 7–9). Details of case selection, age-sex matching of case-controls, and transparency in data and method were reported in most of the studies (Additional File 1: Table S4). The risk of bias assessment for the 19 studies suggested low risk for study design for the majority of the studies (not shown). However, significant publication bias was observed after assessment using funnel plot (Additional File 1: Figure S7-S9) and Egger's test ($p < 0.05$).

Discussion

The present systematic review and meta-analysis aimed to identify and analyze tear-fluid-based diabetic ocular complication biomarkers that are currently reported in the literature. The 19 studies encompassing 1413 participants (from three continents) across 3 groups, indicated that concentrations of TNF-α, VEGF, IL-6, and IL-8 increased in individuals with ocular complications of diabetes (Figs. 2 and 4), with TNF-α, IL-6 and VEGF demonstrating consistent and statistically significant elevated concentrations in the tear-fluid of Complications group as compared to the Control or Diabetes groups. Comparison of TNF-α and VEGF concentrations between the Controls and Diabetes groups did not yield any statistical significance (Fig. 3), indicating their specificity in tear-fluid to diabetic ocular complications. Lactoferrin, lysozyme, and IL-1RA were lower in the Complications group; however, only lactoferrin demonstrated significance between the comparisons (Fig. 2, Additional File 1: Figure S1).

The biomarkers highlighted in this meta-analysis are a combination of proteins and cytokines. Increased concentration of VEGF in the retina is one of the most established biomarkers for ocular complications such as DR, where increased VEGF results in neovascularisation [65]. We also observed a higher concentration of VEGF in tear-fluid from individuals in Complications groups. The majority of the remaining biomarkers are cytokines; IL-6 is a pro-inflammatory cytokine that is produced in response to an infection or tissue damage [66] and is often associated with chronic injury, more specifically, ocular damage [67]. IL-8 is also known to be involved in

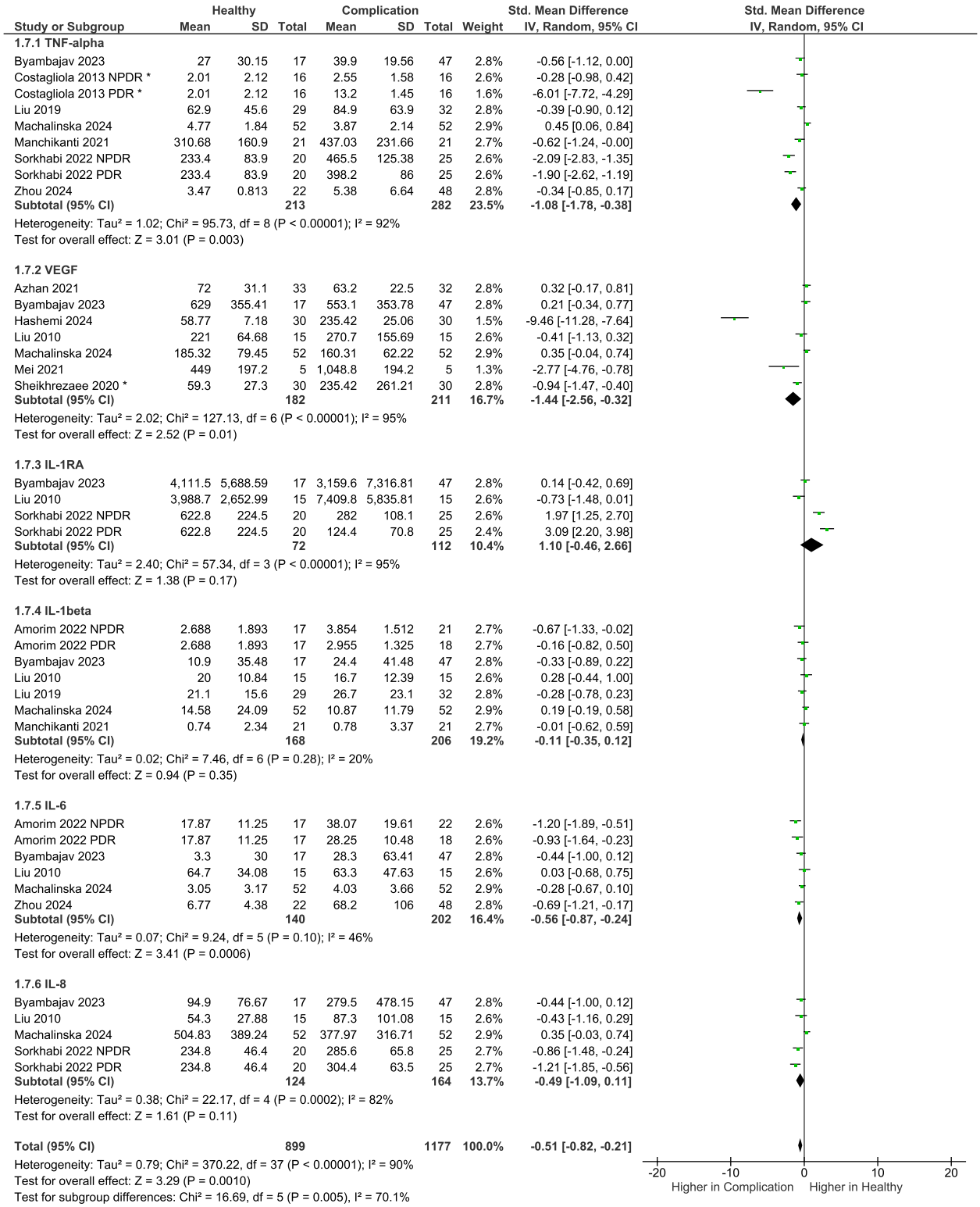

Fig. 2 Biomarker concentrations reported from each study compared between Control group and Complications group. Data represented as standardized mean difference (SMD) have been divided into two groups: one with healthy control participants and the other of individuals with clinical signs of diabetes and ocular complications. Both groups show concentrations for TNF-α, VEGF, IL-1RA, IL-1β, IL-6, and IL-8. Studies that present the concentrations of these markers for NPDR as well as PDR are listed separately. IV inverse variance, CI confidence interval, NPDR non-proliferative diabetic retinopathy, PDR proliferative diabetic retinopathy. Asterisk indicates study data was extracted using WebPlotDigitzer

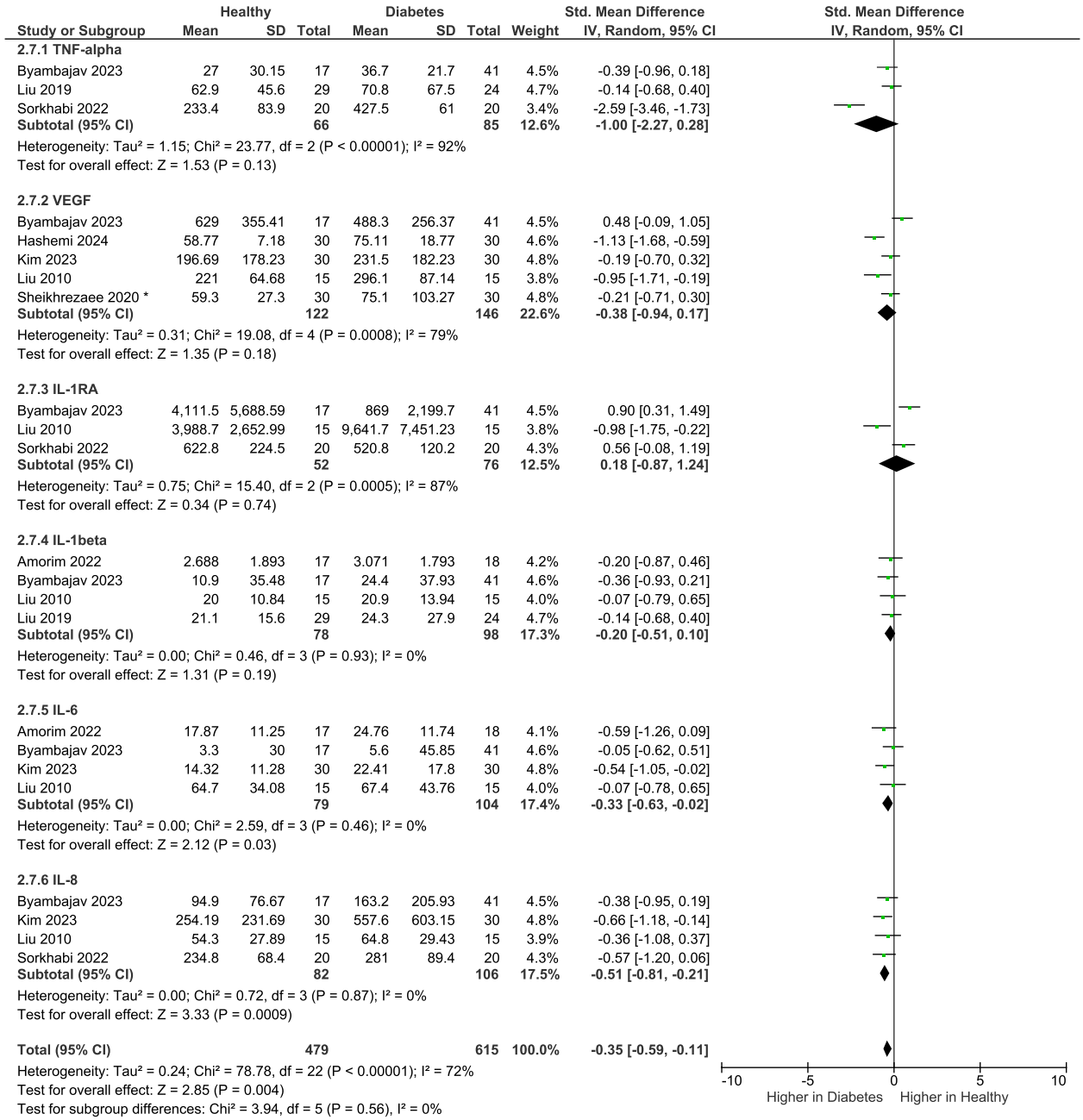

Fig. 3 Biomarker concentrations reported from each study compared between the Control group and the Diabetes group. Data represented as standardized mean difference (SMD) have been divided into two groups: one with healthy control participants, and the other showing clinical signs of diabetes with no noted ocular complications. Both groups show concentrations for TNF-α, VEGF, IL-1RA, IL-1β, IL-6, and IL-8. IV inverse variance, CI confidence interval, Asterisk indicates study data was extracted using WebPlotDigitzer

ocular inflammation [68]. IL-1β, another pro-inflammatory cytokine, is secreted in response to injury or damage to mediate inflammation as a host-defense mechanism [69]. Increased concentration of IL-1β in the tear-fluid has been observed in dry eye disease [70]. IL-1RA is known to block the binding of IL-1β to IL-1 receptor 1 (IL-1R1), thus is important in controlling IL-1β activity [71]. TNF-α is also one of the common biomarkers identified in this meta-analysis and is often associated with chronic inflammation as well as insulin resistance [27]. MCP-1 (CCL2) is a potent inflammatory cytokine, and it has been shown to be involved in retinal inflammation

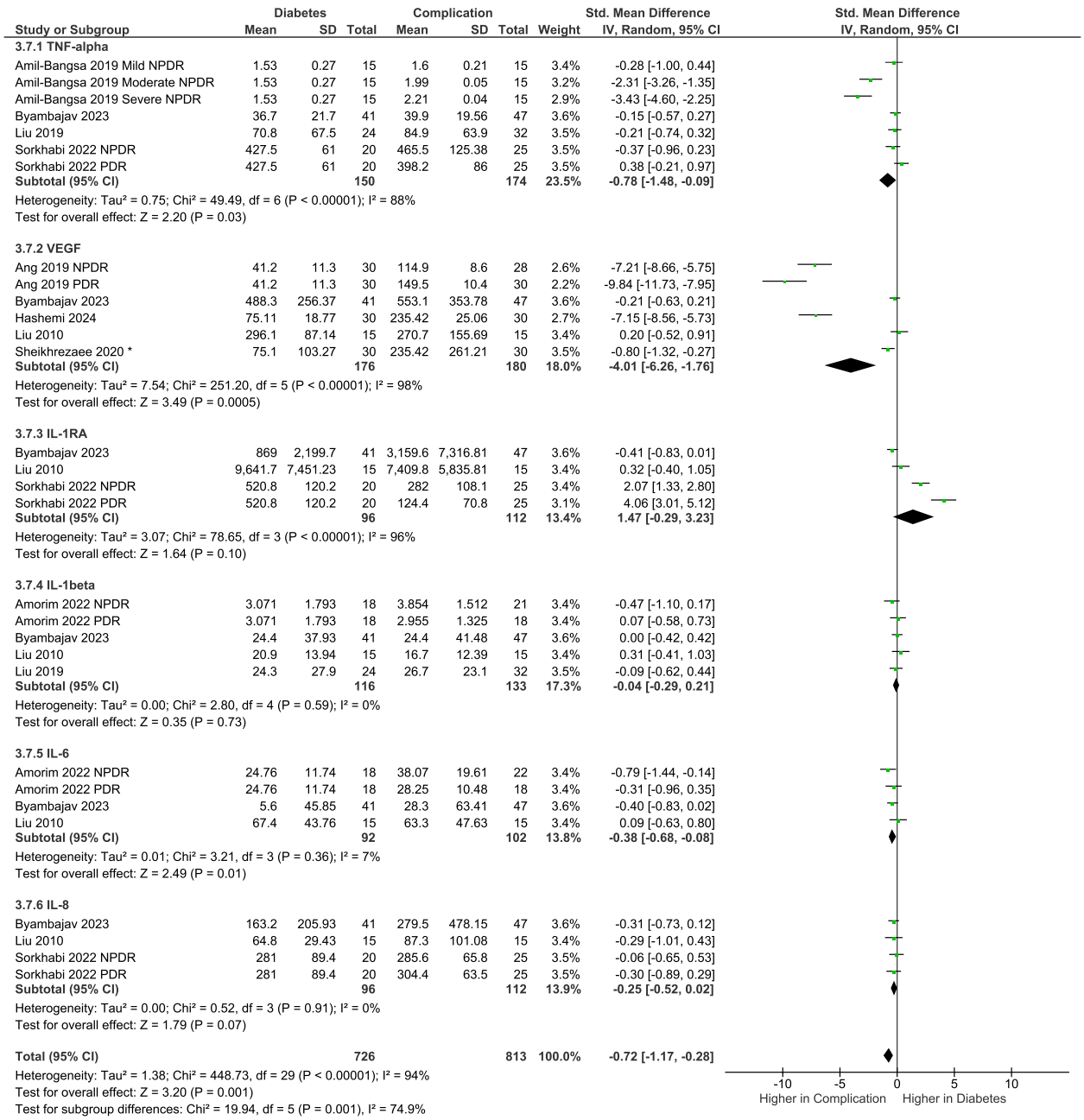

Fig. 4 Biomarker concentrations reported from each study compared between the Diabetes group and the Complications group. Data represented as standardized mean difference (SMD) have been divided into two groups: one with diabetes participants without ocular complications, and the other showing clinical signs of diabetes and ocular complications. Both groups show concentrations for TNF-α, VEGF, IL-1RA, IL-1β, IL-6, and IL-8. Studies that present the concentrations of these markers for NPDR as well as PDR are listed separately. IV inverse variance, CI confidence interval, NPDR non-proliferative diabetic retinopathy, PDR proliferative diabetic retinopathy. Asterisk indicates study data was extracted using WebPlotDigitzer

in diabetes via monocyte and macrophage recruitment and activation [72]. In addition, MCP-1 along with other inflammatory markers (VEGF, TNF-α, IL-1β, and IL-6) was observed to be elevated in the aqueous humor and vitreous fluid of individuals with PDR and diabetic macular edema [72]. Lactoferrin is another potential molecule that has been studied in diabetic ocular complications. There is a meta-analysis on lactoferrin in DR and other ocular complications [23], suggesting lower concentrations in dry eye disease. Our findings corroborate

Table 3 New technologies developed to detect prospective biomarkers in tear fluid

Reference	Technology	Brief description	Target disease	Analyte used
[53]	Flexible electrochemical sensor	A screen-printed carbon electrode was fabricated using polyimide film. The flexible electrode was prepared with $Fe_xCo_yO_4$-rGO. Detection limit: 0.07 µM; sensitivity; 1510 µM cm^{-2} mA^{-1}	Diabetes mellitus	Glucose
[54]	Optoelectrokinetic bead-based immunosensing technique	A combination of bead-based assays and rapid electrokinetic patterning. Limit of detection: 110 pg/mL	Diabetic retinopathy	Lipocalin-1
[61]	Open-well configuration optoelectrokinetic biochip	A combination of bead-based assays and rapid electrokinetic patterning built on an open-form biochip creates more flexibility in sample processing. Limit of detection: 2.9 pg/mL	N/A	Tumor necrosis factor-alpha
[57]	Aptamer triple-helix molecular switch probe	Makes use of electrochemical signal transduction and also uses a CHA/HCR nanomaterial- and enzyme-free signal amplification cascade strategy. Detection limit: 0.87 pM	Diabetic retinopathy	Lipocalin-1
[59]	InflammaDry	A 10-min immunoassay was used to detect levels of matrix metalloproteinase-9 > 40 ng/mL	Glaucoma	Matrix metalloproteinase-9
[60]	Rapid electrokinetic patterning chip	A combination of an optoelectrokinetic platform and a bead-based immunoassay. The limit of detection can be as low as 100 pg/mL	Diabetic retinopathy	Lipocalin-1; vascular endothelial growth factor
[58]	Diagnostic capillary with porous hydrogel	Developed to detect biomarkers in 16 min, the device works by attaching probe particles to capture particles and forming sandwiched immunocomplexes in the presence of the target biomarker. Limit of detection: 1 ng/mL	Diabetic retinopathy	Lipocalin-1
[56]	Immunofluorescence biosensor enhanced with a photonic crystal	Constructed by self-assembled nanoparticles that are coated with a thin layer of gold. Detects trace proteins suspended in tears. Limit of detection: 3 µg/mL	Diabetic retinopathy	Lipocalin-1
[55]	Diffusometric immunosensing technique with grafted gold nanoparticles	Technique was designed to detect small molecules for early disease diagnosis or low-abundance biomarkers. Fluorescent nanosized particles were conjugated with a capture antibody to selectively detect suspended target biomarkers. Limit of detection: 10 pg/mL	Diabetic retinopathy	Tumor necrosis factor-alpha
[37]	Ultrasensitive reusable electrochemical aptasensor	A hybridization chain reaction and CeO_2 nanoparticles were used to create a cascade signal amplification strategy to detect VEGF. Limit of detection: 0.27 fg/mL	Diabetic retinopathy	Vascular endothelial growth factor
[64]	Fiber optic label-free biosensor	The system utilizes a fiber-optic ball resonator, fabricated with a CO2 laser splicer. Limit of detection: 240 ag/mL. The device can detect LCN-1 protein concentrations ranging up to 10 ng/mL	Diabetic retinopathy	Lipocalin-1
[63]	Colorimetric and fluorescent biosensors	Biosensors prepared from polyethylene glycol diacrylate-based inverse opal photonic crystal decorated with graphene oxide. Limit of detection: 6.7×10^{-5} mg/mL	Diabetic retinopathy	Lipocalin-1
[62]	Electrical biosensor chip	A multi-layer paper-based biosensor chip connected with a handheld printed circuit board to monitor the effectiveness of beta-2-microglobuin and lactoferrin in the contrived tear film. Range of detection from 0.001 to 1 ng/mL and 1 to 10 mg/mL, respectively	Dry eye syndrome; diabetic retinopathy	Lactoferrin, beta-2-microglobuin

with these data, wherein we also observe significantly lower lactoferrin in diabetic ocular complications. Lysozyme is an antimicrobial protein (AMP), highly abundant in tears and is implicated in mucosal immunity [73]. The presence of these biomarkers in the tear-fluid is consistent with their function and profiles, indicative of tissue damage in diabetic ocular complications [74]. Exact origin and route of these molecules into tear-fluid is unknown. A logical derivation is that the localized milieu of increased pro-inflammatory cytokines and vascular growth factors during progression to ocular complications contributes to leakage or targeted release (via exosomes/extracellular vesicles) of these biomolecules into the tear-fluid.

At the full-text screening stage, we identified a plethora of other potential biomarkers that were profiled in the tear samples of diabetic ocular complications (Additional File 1: Table S3). These markers were not included for analysis as they did not meet our pre-defined inclusion criteria (actual concentrations reported in at least three studies) but they may hold promise as potential diagnostic tools. LCN-1 is the third most abundant protein in tear-fluid that is primarily responsible for binding to lipids and cholesterol [75]. In DR, concentrations of LCN-1 have been reported to be elevated, compared to healthy individuals [76]. Although LCN-1 was measured in more than three studies, we could not include it in the meta-analysis due to insufficient data.

The two most commonly reported classes of biomarkers were proteins and cytokines, while other biomarker types included metabolites and amino acids, peptides, enzymes, trace metals, glucose, as well as microRNAs/miRNAs (Additional File 1: Table S3). MiRNAs are small non-coding RNAs that regulate gene expression and are emerging as biomarkers for different diseases [77–81]. Our systematic screening identified three studies reporting miRNAs as potential biomarkers for diabetic ocular complication [82–84]; however, they were ineligible for data extraction and meta-analysis due to the lack of actual concentration/expression data.

The limitations of this study are the inadequate number of articles reporting actual concentrations of tear-fluid-based biomarkers for subgroup analyses. The majority of the biomarkers (Additional File 1: Table S3) identified in our search were reported in one or two studies, which severely limited the number of papers that were finally included in this meta-analysis. Another limitation was the high heterogeneity in this meta-analysis. The tear-fluid collection methods, and protein quantification methods (ELISA, LC–MS, and bead-based assays) are noted to produce different sensitivity and specificity values across different reports [85, 86]. In our study, subgroup analysis for tear collection methods (capillary vs Schrimer paper) indicated differences in the I^2 values for some of the markers, suggesting tear collection method can introduce heterogeneity in the results. Studies from different regions of the world may produce context/ethnicity-based bias. Analysis to understand the contribution of different factors towards heterogeneity was not possible due to fewer number of publications in each subgroup. Future studies with larger and more diverse cohort of study participants, along with optimized sample collection methods are needed. While DR can be further classified into non-proliferative (NPDR) and proliferative (PDR) stages, a limited number of studies prevented us to perform a sub-analysis of biomarkers to differentiate between these stages. Underlying confounding factors such as other systemic diseases, ocular or systemic inflammation could lead to the presence of inflammatory markers in the tears. However, we find that the majority of the studies (14 of 19) have strict exclusion criteria where participants with existing active or chronic eye infections, ocular allergies, inflammatory diseases of the eye surface, history of eye surgeries, and systemic inflammation were excluded. Additionally, the majority of the cases and controls in this meta-analysis were matched for age and sex, and several studies were also matched for co-morbidities, smoking, and diabetes duration.

Despite these limitations, this meta-analysis is the first to comprehensively evaluate the effectiveness of tear-fluid-based proteins and cytokines in the diagnosis of ocular complications in diabetes. Through group-wise comparison of study participants from 19 studies, we identified that the tear-fluid concentration of TNF-α and VEGF are significantly different in individuals with ocular complications of diabetes. However, as the heterogeneity (I^2) values were high, future validation on larger cohorts as well as mechanistic understanding of their increased concentration in tear-fluid will be insightful. Additionally, we report newer methodologies that are being developed to assess tear-fluid-based biomarkers (Table 3). Although we did not find any longitudinal cohort study exploring tear biomarkers of diabetic ocular complications, our work provides a framework for undertaking prospective clinical studies to assess the biomarkers found to be significantly dysregulated in this meta-analysis. Tear-fluid provides a non-invasive material for longitudinal biomarker profiling, and therefore, it is important to develop an easy-to-use, portable, and economical platform that captures changes in the levels of such biomarkers.

Conclusions

This is the first meta-analysis identifying a set of tear-fluid-based biomarkers across individuals without (Control), with diabetes, and those with ocular complications of diabetes. This meta-analysis demonstrated that while there are several studies on tear-fluid-based biomarkers, only a few of these measure the same biomarkers using standardized assays. Here we show that TNF-α and VEGF independently or together with other biomarkers have the potential to stratify individuals with ocular complications of diabetes compared to those without any ocular complications (Diabetes only) or those without diabetes (Controls). Future studies could focus on determining the predictive power of these biomarkers and the deployment of point-of-care technologies to facilitate longitudinal and cost-effective assessment of ocular health for risk stratification of those in our remote/rural communities.

Abbreviations

AMP	Antimicrobial protein
BCA	Bicinchoninic acid
CI	Confidence interval
DCN	Diabetic corneal neuropathy
DED	Dry eye disease
DM	Diabetes mellitus
DPN	Diabetic peripheral neuropathy
DR	Diabetic retinopathy
ELISA	Enzyme-linked immunosorbent assay
EV	Extracellular vesicle
HPLC	High-performance liquid chromatography
IL-1 B	Interleukin 1 beta
IL-1RA	Interleukin-1 receptor agonist
IL-6	Interleukin 6
IL-8	Interleukin 8
IV	Inverse variance
LC–MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LCN-1	Lipocalin 1
LTF	Lactoferrin
LZM	Lysozyme
MCP-1	Monocyte chemoattractant protein-1
MeSH	Medical Subject Heading
NOS	Newcastle–Ottawa scale
NPDR	Non-proliferative diabetic retinopathy
OCT	Optical coherence tomography
PDR	Proliferative diabetic retinopathy
PICOS	Participants, Intervention, Comparators, Outcome, Study Design
PRISMA	Preferred Reporting Items for Systematic Reviews And Meta-Analyses
RFP	Retinal fundus photographs
SD	Standard Deviation
SDS-PAGE	Dodium dodecyl-polyacrylamide gel electrophoresis
SMD	Standard mean difference
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TBUT	Tear break up time
TMT	Tandem mass tag
TNF- A	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03855-z.

Additional file 1: Table S1: Previously published relevant meta-analyses and systematic reviews that identify biomarkers from tear-fluid in a range of diseases. Table S2: Database Search Strategies. Table S3: Tear-fluid based biomolecules reported in articles that were included at the full-text screening stage. Table S4: Quality assessment of the articles included in the meta-analysis using the Newcastle–Ottawa Scale of the case–control studies with quantitative outcome. Figure S1: Comparison of LTF and LZM in Control vs Complications groups. Figure S2: Comparison of MCP-1 in Control vs Diabetes groups. Figures S3-S5: Leave-one-out sensitivity analysis for comparisons between Control, Diabetes, and Complications groups. Figure S6: Subgroup analysis of examined biomarkers in Control vs Complications comparison. Figures S7-S9: Funnel plot and Egger's test between Control, Diabetes, and Complications groups.

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Authors' contributions

MVJ, MAC and AAH conceptualised the study; MP, NHTP, WKMW, HPH and MVJ independently performed the article search and data extraction before cross-validation. MP, NHTP, HPH, PSK and MVJ contributed to data interpretation and analysis; MP wrote the first draft; all authors edited the manuscript; MVJ, MAC and AAH finalised the manuscript draft. MVJ and AAH are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Data availability

This manuscript reports meta-analysis of published data, which will be made available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publications

Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2023;402(10397):203–34.
2. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: an overview. Avicenna J Med. 2020;10(4):174–88.
3. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34 Suppl 1(Suppl 1), S62–9.
4. Teo ZL, Tham YC, Yu M, Chee ML, Rim TH, Cheung N, et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. Ophthalmology. 2021;128(11):1580–91.
5. Han SB, Yang HK, Hyon JY. Influence of diabetes mellitus on anterior segment of the eye. Clin Interv Aging. 2019;14:53–63.
6. Ferrara M, Loda A, Coco G, Grassi P, Cestaro S, Rezzola S, et al. Diabetic retinopathy: soluble and imaging ocular biomarkers. J Clin Med. 2023;12(3):912.
7. Jenkins AJ, Joglekar MV, Hardikar AA, Keech AC, O'Neal DN, Januszewski AS. Biomarkers in diabetic retinopathy. Rev Diabet Stud. 2015;12(1–2):159–95.
8. Rizvi A, Rizvi F, Lalakia P, Hyman L, Frasso R, Sztandera L, et al. Is artificial intelligence the cost-saving lens to diabetic retinopathy screening in low- and middle-income countries? Cureus. 2023;15(9): e45539.
9. Hu W, Joseph S, Li R, Woods E, Sun J, Shen M, et al. Population impact and cost-effectiveness of artificial intelligence-based diabetic retinopathy screening in people living with diabetes in Australia: a cost effectiveness analysis. eClinicalMedicine. 2024;67:102387.
10. Vujosevic S, Aldington SJ, Silva P, Hernandez C, Scanlon P, Peto T, et al. Screening for diabetic retinopathy: new perspectives and challenges. Lancet Diabetes Endocrinol. 2020;8(4):337–47.
11. Abou Taha A, Dinesen S, Vergmann AS, Grauslund J. Present and future screening programs for diabetic retinopathy: a narrative review. Int J Retina Vitreous. 2024;10(1):14.
12. Hagan S, Martin E, Enríquez-de-Salamanca A. Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalised medicine. Epma J. 2016;7(1):15.
13. Kaštelan S, Orešković I, Bišćan F, Kaštelan H, Gverović AA. Inflammatory and angiogenic biomarkers in diabetic retinopathy. Biochem Med (Zagreb). 2020;30(3): 030502.
14. Suárez-Cortés T, Merino-Inda N, Benitez-Del-Castillo JM. Tear and ocular surface disease biomarkers: a diagnostic and clinical perspective for ocular allergies and dry eye disease. Exp Eye Res. 2022;221: 109121.
15. Wong WKM, Polkamp M, Farr RJ, Kunte PS, Hardikar HP, Yajnik CS, et al. MicroRNA profiling from tears as a potential non-invasive method for early detection of diabetic retinopathy. Methods Mol Biol. 2023;2678:117–34.
16. Ponzini E. Tear biomarkers. Adv Clin Chem. 2024;120:69–115.
17. Król-Grzymała A, Sienkiewicz-Szłapka E, Fiedorowicz E, Rozmus D, Cieślińska A, Grzybowski A. Tear biomarkers in Alzheimer's and Parkinson's diseases, and multiple sclerosis: implications for diagnosis (systematic review). Int J Mol Sci. 2022;23(17):10123.
18. Khanna RK, Catanese S, Emond P, Corcia P, Blasco H, Pisella PJ. Metabolomics and lipidomics approaches in human tears: a systematic review. Surv Ophthalmol. 2022;67(4):1229–43.
19. Aydin E, Gokhale M, Azizoglu S, Suphioglu C. To see or not to see: a systematic review of the importance of human ocular surface cytokine biosignatures in ocular allergy. Cells. 2019;8(6):620.
20. Poon SHL, Cheung JJ, Shih KC, Chan YK. A systematic review of multimodal clinical biomarkers in the management of thyroid eye disease. Rev Endocr Metab Disord. 2022;23(3):541–67.
21. Roda M, Corazza I, Bacchi Reggiani ML, Pellegrini M, Taroni L, Giannaccare G, et al. Dry eye disease and tear cytokine levels-a meta-analysis. Int J Mol Sci. 2020;21(9):3111.
22. Navel V, Malecaze J, Pereira B, Baker JS, Malecaze F, Sapin V, et al. Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis. Acta Ophthalmol. 2021;99(6):e777–94.
23. Ponzini E, Scotti L, Grandori R, Tavazzi S, Zambon A. Lactoferrin concentration in human tears and ocular diseases: a meta-analysis. Invest Ophthalmol Vis Sci. 2020;61(12):9.
24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372: n71.
25. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014;14(1):135.
26. Amorim M, Martins B, Caramelo F, Gonçalves C, Trindade G, Simão J, et al. Putative biomarkers in tears for diabetic retinopathy diagnosis. Front Med (Lausanne). 2022;9: 873483.
27. Costagliola C, Romano V, De Tollis M, Aceto F, dell'Omo R, Romano MR, et al. TNF-alpha levels in tears: a novel biomarker to assess the degree of diabetic retinopathy. Mediators Inflamm. 2013;2013:629529.
28. Sheikhrezaee M, Alizadeh MR, Abediankenari S. The tear VEGF and IGFBP3 in healthy and diabetic retinopathy. Int Diabetes Dev Ctries. 2020;40(1):93–8.
29. Rohatgi A. WebPlotDigitizer Pacifica, CA, USA 2022;4.6. Available from: https://automeris.io/WebPlotDigitizer.
30. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000.
31. Julian PTH, Douglas GA, Peter CG, Peter J, David M, Andrew DO, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343: d5928.
32. Jonathan ACS, Miguel AH, Barnaby CR, Jelena S, Nancy DB, Meera V, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;355: i4919.
33. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–34.
34. Balduzzi S, Rücker G, G S. How to perform a meta-analysis with R: a practical tutorial. Evid-Based Mental Health. 2019;22:153–60.
35. Harrer M, Cuijpers P, Furukawa T, D ED. dmetar: companion R package for the guide 'doing meta-analysis in R. 2019.
36. Harrer M, Cuijpers P, Furukawa TA, Ebert DD. Doing meta-analysis with R: a hands-on guide. 1st ed. Boca Raton, FL and London: Chapman & Hall/CRC Press; 2021. p. 2021.
37. Mei C, Pan L, Xu W, Xu H, Zhang Y, Li Z, et al. An ultrasensitive reusable aptasensor for noninvasive diabetic retinopathy diagnosis target on tear biomarker. Sens Actuators, B Chem. 2021;345: 130398.
38. Manchikanti V, Kasturi N, Rajappa M, Gochhait D. Ocular surface disorder among adult patients with type II diabetes mellitus and its correlation with tear film markers: a pilot study. Taiwan J Ophthalmol. 2021;11(2):156–60.
39. Byambajav M, Collier A, Shu X, Hagan S. Tear fluid biomarkers and quality of life in people with type 2 diabetes and dry eye disease. Metabolites. 2023;13(6):733.
40. Liu R, Ma B, Gao Y, Ma B, Liu Y, Qi H. Tear inflammatory cytokines analysis and clinical correlations in diabetes and nondiabetes with dry eye. Am J Ophthalmol. 2019;200:10–5.
41. Sorkhabi R, Ahoor Mh, Ghorbani Haghjo A, Tabei E, Taheri N. Assessment of tear inflammatory cytokines concentration in patients with diabetes with varying severity of involvement. Exp Eye Res. 2022;224: 109233.
42. Liu J, Shi B, He S, Yao X, Willcox MD, Zhao Z. Changes to tear cytokines of type 2 diabetic patients with or without retinopathy. Mol Vis. 2010;16:2931–8.

43. Azhan A, Zunaina E, Mahaneem M, Siti-Azrin AH. Comparison of VEGF level in tears post phacoemulsification between non-proliferative diabetic retinopathy and non-diabetic patients. J Diabetes Metab Disord. 2021;20(2):2073–9.
44. Kim AY, Moon JY, Jun RM, Kim HJ, Han KE. Ocular surface and tear cytokine changes after cataract surgery in patients with type 2 diabetes. Ocul Immunol Inflamm. 2023;31(8):1615–22.
45. Zhou T, Dou Z, Cai Y, Zhu D, Fu Y. Tear fluid progranulin as a noninvasive biomarker for the monitoring of corneal innervation changes in patients with type 2 diabetes mellitus. Transl Vis Sci Technol. 2024;13(7): 9.
46. Hashemi H, Ahmadi H, Rostami Z, Alishahi A, Heidari Z. The role of endothelial growth factor and tear levels in diabetic retinopathy in type 2 diabetes. Int Ophthalmol. 2024;44(1):143.
47. Machalińska A, Kuligowska A, Ziontkowska-Wrzałek A, Stroynowska B, Pius-Sadowska E, Safranow K, et al. The severity of diabetic retinopathy corresponds with corneal nerve alterations and ocular discomfort of the patient. Int J Mol Sci. 2024;25(11):6072.
48. Amil-Bangsa NH, Mohd-Ali B, Ishak B, Abdul-Aziz CNN, Ngah NF, Hashim H, et al. Total protein concentration and tumor necrosis factor α in tears of nonproliferative diabetic retinopathy. Optom Vis Sci. 2019;96(12):934–9.
49. Stolwijk TR, Kuizenga A, Van Haeringen NJ, Kijlstra A, Oosterhuis JA, Van Best JA. Analysis of tear fluid proteins in insulin-dependent diabetes mellitus. Acta Ophthalmol. 1994;72(3):357–62.
50. Zou X, Zhang P, Xu Y, Lu L, Zou H. Quantitative proteomics and weighted correlation network analysis of tear samples in type 2 diabetes patients complicated with dry eye. Proteomics Clin Appl. 2020;14(4): e1900083.
51. Yu L, Chen X, Qin G, Xie H, Lv P. Tear film function in type 2 diabetic patients with retinopathy. Ophthalmologica. 2008;222(4):284–91.
52. Ang WJ, Zunaina E, Norfadzillah AJ, Raja-Norliza RO, Julieana M, Ab-Hamid SA, et al. Evaluation of vascular endothelial growth factor levels in tears and serum among diabetic patients. PLoS ONE. 2019;14(8): e0221481.
53. Zhou F, Zhao H, Chen K, Cao S, Shi Z, Lan M. Flexible electrochemical sensor with Fe/Co bimetallic oxides for sensitive analysis of glucose in human tears. Anal Chim Acta. 2023;1243: 340781.
54. Wang JC, Ku HY, Chen TS, Chuang HS. Detection of low-abundance biomarker lipocalin 1 for diabetic retinopathy using optoelectrokinetic bead-based immunosensing. Biosens Bioelectron. 2017;89(Pt 2):701–9.
55. Chuang HS, Chen YJ, Cheng HP. Enhanced diffusometric immunosensing with grafted gold nanoparticles for detection of diabetic retinopathy biomarker tumor necrosis factor-α. Biosens Bioelectron. 2018;101:75–83.
56. Chen LY, Hsu SM, Wang JC, Yang TH, Chuang HS. Photonic crystal enhanced immunofluorescence biosensor integrated with a lateral flow microchip: toward rapid tear-based diabetic retinopathy screening. Biomicrofluidics. 2023;17(4): 044102.
57. Yao J, Liu Y, Jiang B, Yuan R, Xiang Y. An aptamer triple helix molecular switch for sensitive electrochemical assay of lipocalin 1 biomarker via dual signal amplifications. Analyst. 2023;148(12):2739–44.
58. Guzman J, Hsu SM, Chuang HS. Colorimetric diagnostic capillary enabled by size sieving in a porous hydrogel. Biosensors (Basel). 2020;10(10):130.
59. Kim DW, Seo JH, Lim S-H. Evaluation of ocular surface disease in elderly patients with glaucoma: expression of matrix metalloproteinase-9 in tears. Eye. 2021;35(3):892–900.
60. Wang J-Y, Kwon J-S, Hsu S-M, Chuang H-S. Sensitive tear screening of diabetic retinopathy with dual biomarkers enabled using a rapid electrokinetic patterning platform. Lab Chip. 2020;20(2):356–62.
61. Chen W-L, Jayan M, Kwon J-S, Chuang H-S. Facile open-well immunofluorescence enhancement with coplanar-electrodes-enabled optoelectrokinetics and magnetic particles. Biosens Bioelectron. 2021;193: 113527.
62. Khan MS, Dighe K, Wang Z, Daza EA, Schwartz-Duval AS, Rowley CP, et al. Label-free detection of lactoferrin and beta-2-microglobuin in contrived tear film using a low-cost electrical biosensor chip. 2017 IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT). 2017:72–5.
63. Phong PH, Chuang H-S, Thi Thuong D, Sang NN, Thi Ha Lien N, Nghia NT, et al. Graphene oxide-decorated hyrogel inverse opal photonic crystal improving colorimetric and fluorescent responses for rapid detection of lipocalin-1. Photonics and Nanostructures - Fundamentals and Applications. 2024;58: 101237.
64. Gomez A, Myrkhiyeva Z, Tilegen M, Pham T, Bekmurzayeva A, Tosi D. Optical fiber ball resonator biosensor as a platform for detection of diabetic retinopathy biomarkers in tears. IEEE Sens J. 2024;24:11127–35.
65. Youngblood H, Robinson R, Sharma A, Sharma S. Proteomic biomarkers of retinal inflammation in diabetic retinopathy. Int J Mol Sci. 2019;20(19):4755.
66. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10): a016295.
67. Shrestha GS, Vijay AK, Stapleton F, Carnt NA. The effect of collection method on tear interleukin-6 levels in healthy individuals: a pilot study. Investigative Ophthalmology, Visual Science. 2019;60(9):5370-.
68. Ghasemi H, Ghazanfari T, Yaraee R, Faghihzadeh S, Hassan ZM. Roles of IL-8 in ocular inflammations: a review. Ocul Immunol Inflamm. 2011;19(6):401–12.
69. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev. 2011;22(4):189–95.
70. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci. 2001;42(10):2283–92.
71. Hallegua DS, Weisman MH. Potential therapeutic uses of interleukin 1 receptor antagonists in human diseases. Ann Rheum Dis. 2002;61(11):960–7.
72. Taghavi Y, Hassanshahi G, Kounis NG, Koniari I, Khorramdelazad H. Monocyte chemoattractant protein-1 (MCP-1/CCL2) in diabetic retinopathy: latest evidence and clinical considerations. J Cell Commun Signal. 2019;13(4):451–62.
73. Hanstock HG, Edwards JP, Walsh NP. Tear lactoferrin and lysozyme as clinically relevant biomarkers of mucosal immune competence. Front Immunol. 2019;10:1178.
74. Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic retinopathy: vascular and inflammatory disease. J Diabetes Res. 2015;2015:582060.
75. Kim HJ, Kim PK, Yoo HS, Kim CW. Comparison of tear proteins between healthy and early diabetic retinopathy patients. Clin Biochem. 2012;45(1):60–7.
76. Gao S, Zhang S, Sun X, Zheng X, Wu J. Fluorescent aptasensor based on G-quadruplex-assisted structural transformation for the detection of biomarker lipocalin 1. Biosens Bioelectron. 2020;169: 112607.
77. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology. 2018;9:402.
78. Ratti M, Lampis A, Ghidini M, Salati M, Mirchev MB, Valeri N, et al. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: first steps from bench to bedside. Target Oncol. 2020;15(3):261–78.
79. Ranganathan K, Sivasankar V. MicroRNAs - Biology and clinical applications. J Oral Maxillofac Pathol. 2014;18(2):229–34.
80. Joglekar MV, Parekh VS, Hardikar AA. New pancreas from old: microregulators of pancreas regeneration. Trends Endocrinol Metab. 2007;18(10):393–400.
81. Wong WKM, Sorensen AE, Joglekar MV, Hardikar AA, Dalgaard LT. Non-coding RNA in pancreas and beta-cell development. Noncoding RNA. 2018;4(4):41.
82. Pinazo-Durán MD, Zanón-Moreno V, Lleó-Perez A, García-Medina JJ, Galbis-Estrada C, Roig-Revert MJ, et al. Genetic systems for a new approach to risk of progression of diabetic retinopathy. Arch Soc Esp Oftalmol. 2016;91(5):209–16.
83. Chan HW, Yang B, Wong W, Blakeley P, Seah I, Tan QSW, et al. A pilot study on MicroRNA profile in tear fluid to predict response to anti-VEGF treatments for diabetic macular edema. J Clin Med. 2020;9(9):2920.
84. Torimura A, Kanei S, Shimizu Y, Baba T, Uotani R, Sasaki S-i, et al. Profiling miRNAs in tear extracellular vesicles: a pilot study with implications for diagnosis of ocular diseases. Japanese Journal of Ophthalmology. 2024;68(1):70–81.
85. Nattinen J, Aapola U, Jylha A, Vaajanen A, Uusitalo H. Comparison of capillary and Schirmer strip tear fluid sampling methods using SWATH-MS proteomics approach. Transl Vis Sci Technol. 2020;9(3): 16.
86. You J, Willcox MD, Madigan MC, Wasinger V, Schiller B, Walsh BJ, et al. Tear fluid protein biomarkers. Adv Clin Chem. 2013;62:151–96.

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