Increased vitreal levels of interleukin-10 in diabetic retinopathy: a Meta-analysis

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Abstract

- **AIM:** To conduct a Meta-analysis for the change of interleukin-10 (IL-10) concentration in vitreous samples of patients with diabetic retinopathy (DR).
- **METHODS:** Systemic search for literature was conducted from the databases of PubMed, Web of Science and Cochrane Library by August 2019. Statistical analyses including standard mean difference (SMD) and its 95% confidence interval (CI) were performed by using RevMan 5.3 software.
- **RESULTS:** Totally 194 studies were screened and finally 11 studies were included in the Meta-analysis. The concentration of IL-10 in the DR group was higher than in the control group ($P=0.003$, SMD: 0.77, 95%CI: 0.25-1.28). Significant heterogeneity was found among all studies ($P<0.00001$, $I^2=92$%). The subgroup analysis showed that the concentration of IL-10 increased in vitreous samples from patients with DR compared to the non-DR controls ($P=0.004$, SMD: 1.44, 95%CI: 0.46-2.42). Moreover, the concentration of IL-10 in samples of proliferative diabetic retinopathy (PDR) patients was significantly higher than that of non-proliferative diabetic retinopathy (NPDR) patients ($P=0.01$, SMD: 0.61, 95%CI: 0.13-1.08).
- **CONCLUSION:** The vitreal concentration of IL-10 is significantly increased in patients with DR. Further studies are needed to reveal the mechanisms of IL-10 in DR.
- **KEYWORDS:** diabetic retinopathy; interleukin-10; Meta-analysis; vitreal concentration

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INTRODUCTION

Diabetic retinopathy (DR) is one of the governing causes of visual impairment or even blindness$^{[1-3]}$, and diabetes patients have 2.4 times higher risk of blindness than those without diabetes$^{[4]}$. DR could be divided into proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy (NPDR) based on the stage of development and severity$^{[5]}$. Changes in the structure and function of retinal microcirculation are the basic pathological processes of DR$^{[6]}$. The blood-retinal barrier could be destroyed by high levels of cytokines and inflammatory mediators$^{[7]}$. Thus, inflammation is considered to be mainly involved in DR pathogenesis. The normal expression of vascular endothelial growth factor (VEGF) could maintain the functional and structural homeostasis of cells in the retina. However, under the influence of pathological factors, overexpression of VEGF may cause retinal angiogenesis$^{[7-9]}$. It has been proved that intravitreal injection of anti-VEGF agents could lead to the inhibition of retinal neovascularization$^{[10]}$. Therefore, it is critical to understand the relationship between pathological neovascularization and microvascular degeneration in the pathophysiology of DR, especially for developing new drugs to improve therapeutic efficacy$^{[11]}$.

It is known that the expression of inflammatory mediators are involved in the progression of DR$^{[12]}$. For instance, it has been indicated that the level of interleukin-6 (IL-6) generally increased in patients with DR, and also identified the association of the increased levels and the severity of DR$^{[13]}$. Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine that suppresses T helper (Th) 1 responses and enhances Th2 responses$^{[14]}$. IL-10 has been proved to enhance pathological retinal angiogenesis via regulation of macrophage response to hypoxia$^{[15]}$. IL-10 and its downstream STAT3 signaling activity play a conducive role in regulating the phenotype of aging macrophage in the eye, mainly promoting M2 polarization and new blood vessel formation$^{[16]}$. An early study revealed that
IL-10 promotes laser-induced choroidal neovascularization (CNV) by suppressing macrophage recruitment and infiltration to neovascular complexes\[^{17}\]. Interestingly, Wu et al.\[^{18}\] revealed that IL-10 only inhibits VEGF that generated by M1 macrophages, not M2 phenotype, suggested that macrophage polarization and hypoxia might be involved in the angiogenic regulation by IL-10. There is also a study showing that IL-10 inhibits inflammation, and suppresses retinal pigment epithelial cells proliferation and migration by regulating VEGF in rhegmatogenous retinal detachment\[^{19}\].

Therefore, the role of IL-10 in DR belongs to complicated immunological mechanisms and remains unclear. In this study, we assess the relationship between DR and IL-10 concentration in different kinds of samples from patients with DR and controls through Meta-analysis, and aim to provide a therapeutic target for treating DR patients.

**MATERIALS AND METHODS**

**Search Strategy** Comprehensive literature searched from PubMed, Web of Science and Cochrane Library (August 2, 2019), by using the keywords: “IL-10” OR “IL10” OR “IL 10” OR “interleukin-10” OR “interleukin10” OR “interleukin 10” connected with “diabetic retinopathy”. We only selected articles written in English. We also evaluated other relevant references cited in the qualified literature.

**Criteria of Inclusion and Exclusion** We contained qualified literature in this Meta-analysis from the following aspects: 1) the investigation must include the DR group and the control group; 2) IL-10 concentration is available; 3) experiment based on human samples; 4) the literature is presented in English. We excluded the literature according to the following aspects: 1) the studies does not provide mean and standard deviation (SD) even more send an email asking for no results; 2) not find full-text; 3) literature that does not indicate cytokine concentration; 4) the articles provide incomplete data; 5) died sample; 6) previous surgical treatment in vitreous.

Besides, patients and controls with ocular diseases such as uveitis which might largely influence the experimental results were excluded in the Meta-analysis.

**Quality Assessment and Data Extraction** Two authors (Tan W and Zou JL) respectively extracted the data from the following aspects: first author, publish year, country, types of diabetic mellitus (DM), sample source, study methods, IL-10 concentration, ages, gender ratio. Two reviewers (Tan W and Zou JL) respectively assess all the studies by using the Newcastle Ottawa Scale (NOS)\[^{20}\]. The NOS system is composed of 3 aspects, including selection (4 points), comparability (2 points), and exposure (3 points). The higher scores stand for better qualities. We only choose articles that are higher than 5 points for further analysis.

**RESULTS**

**Literature Selection** The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines\[^{21}\]. A total of 194 articles were included, from PubMed, Web of Science and Cochrane Library (August 2, 2019). After excluding 53 duplicates, we screened the titles and abstracts, and excluded 75 articles. Then, according to full-text, 55 articles were excluded. Finally, we obtained 11 studies for Meta-analysis. The process of selection was summarized in the flowchart (Figure 1).

**Characteristics and Quality Assessment of the Studies** Eleven studies were summarized and analyzed in Table 1, including gender, age, country, DM type and sample origin. The samples research method basically includes multiplex
bead immunoassay, enzyme-linked immunoassay, the Bio-
Plex kit, etc. Using NOS evaluate the quality assessment of
all studies in Table 2. Six studies [22-27] scored 6, 3 studies
[28-30] scored 7, 2 studies [31-32] scored 8. Three studies used samples
from serum [27,31-32], 2 studies from plasma [23,29], 8 studies from
vitreous [22-28,31] and 1 from aqueous humor [30].

Meta-analysis of IL-10 Concentration in Patients with DR

Based on all the included studies, the concentration of
IL-10 in samples between the DR group and the control group
showed significant heterogeneity (P<0.00001, I²=92%), and
we selected the random-effect model. The Meta-analysis
(Figure 2) observed that the concentration of IL-10 in the DR
group was significantly higher compared to the control group
(SMD: 0.77, 95%CI: 0.25 to 1.28, P=0.003).

Table 1 Characteristics of the included studies in the Meta-analysis

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>DM type</th>
<th>Sample</th>
<th>Age (y)</th>
<th>Gender (M, F)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromberg-White</td>
<td>2013</td>
<td>USA</td>
<td>NA</td>
<td>Vitreous</td>
<td>58±11</td>
<td>22, 21</td>
<td>DR 43, Control 29</td>
</tr>
<tr>
<td>Chernykh</td>
<td>2015</td>
<td>Russia</td>
<td>NA</td>
<td>Vitreous</td>
<td>50±3.2</td>
<td>16, 22</td>
<td>DR 38, Control 25</td>
</tr>
<tr>
<td>Kaviarasan</td>
<td>2015</td>
<td>India</td>
<td>2</td>
<td>Serum</td>
<td>55±9.08</td>
<td>35, 26</td>
<td>DR 61, Control 57</td>
</tr>
<tr>
<td>Koskela</td>
<td>2013</td>
<td>Finland</td>
<td>Both</td>
<td>Vitreous</td>
<td>59.4±14.3</td>
<td>17, 21</td>
<td>DR 38, Control 16</td>
</tr>
<tr>
<td>Lee</td>
<td>2008</td>
<td>Korea</td>
<td>2</td>
<td>Plasma</td>
<td>59.87±9.00</td>
<td>13, 15</td>
<td>DR 28, Control 46</td>
</tr>
<tr>
<td>Ozturk</td>
<td>2009</td>
<td>Turkey</td>
<td>2</td>
<td>Serum</td>
<td>62.09±7.26</td>
<td>39, 56</td>
<td>DR 95, Control 59</td>
</tr>
<tr>
<td>Suzuki</td>
<td>2011</td>
<td>Japan</td>
<td>NA</td>
<td>Vitreous</td>
<td>57±13.6</td>
<td>25, 31</td>
<td>DR 56, Control 40</td>
</tr>
<tr>
<td>Takeuchi</td>
<td>2015</td>
<td>Japan</td>
<td>2</td>
<td>Vitreous</td>
<td>57.0±13.7</td>
<td>18, 27</td>
<td>DR 25, Control 53</td>
</tr>
<tr>
<td>Wu</td>
<td>2017</td>
<td>China</td>
<td>2</td>
<td>Aqueous humor</td>
<td>66.48</td>
<td>13, 16</td>
<td>DR 29, Control 32</td>
</tr>
<tr>
<td>Xu</td>
<td>2015</td>
<td>China</td>
<td>NA</td>
<td>Vitreous</td>
<td>52.12±1.474</td>
<td>15, 19</td>
<td>DR 34, Control 37</td>
</tr>
<tr>
<td>Yabanoğlu</td>
<td>2014</td>
<td>Turkey</td>
<td>2</td>
<td>Serum</td>
<td>58±10.5</td>
<td>6, 11</td>
<td>DR 17, Control 9</td>
</tr>
</tbody>
</table>

DM: Diabetic mellitus; DR: Diabetic retinopathy; NA: Not available.

Table 2 Newcastle-Ottawa scale scores of the included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Comparability</th>
<th>Exposure</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromberg-White et al, 2013</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Chernykh et al, 2015</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Kaviarasan et al, 2015</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Koskela et al, 2013</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Lee et al, 2008</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Ozturk et al, 2009</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Suzuki et al, 2011</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Takeuchi et al, 2015</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Wu et al, 2017</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Xu et al, 2015</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Yabanoğlu et al, 2014</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Aqueous humor samples had significant difference (SMD: 0.97, 95%CI: 0.44 to 1.50, P=0.0004), but there was only one study included (Figure 3B). On the other hand, no significant difference was found in serum (SMD: 0.11, 95%CI: -0.13 to
Intravitreal IL-10 level of diabetic retinopathy

Table 3 Subgroup analyses according to sample source and DR type

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Sample source</th>
<th>n</th>
<th>DR</th>
<th>Control</th>
<th>SMD (95%CI)</th>
<th>P</th>
<th>Test of heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SMD (95%CI)</td>
<td></td>
<td>P</td>
<td>Test of heterogeneity</td>
<td></td>
</tr>
<tr>
<td>Sample source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous</td>
<td>306</td>
<td>213</td>
<td>1.44 (0.46, 2.42)</td>
<td>0.004</td>
<td>95</td>
<td>&lt;0.00001</td>
<td></td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>29</td>
<td>32</td>
<td>0.97 (0.44, 1.50)</td>
<td>0.0004</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>162</td>
<td>119</td>
<td>0.11 (-0.13, 0.35)</td>
<td>0.37</td>
<td>0</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>82</td>
<td>44</td>
<td>-0.16 (-0.84, 0.52)</td>
<td>0.64</td>
<td>69</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>DR type</td>
<td>PDR, NPDR</td>
<td>92</td>
<td>0.61 (0.13, 1.08)</td>
<td>0.01</td>
<td>58</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

SMD: Standard mean difference; DR: Diabetic retinopathy; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy.

0.35, P=0.37) and plasma (SMD: -0.16, 95%CI: -0.84 to 0.52, P=0.64) samples (Figure 3C, 3D). Significant heterogeneity was existed in the analyses of vitreous (P<0.0001, I²=95%) and plasma (P=0.07, I²=69%), but not in the analysis of serum (P=0.94, I²=0). Data of patients with DR from 4 studies can be subdivided into PDR and NPDR groups (Figure 4). According to the Meta-analysis, IL-10 concentration was upregulated in PDR patients compared with NPDR patients (SMD: 0.61, 95%CI: 0.13 to 1.08, P=0.01), and significant heterogeneity was existed (P=0.07, I²=58%).

Assessment of Publication Bias Publication bias was firstly assessed by a funnel plot (Figure 5), which was not symmetry. Then we estimated by Begg’s test and Egger’s test. The results showed there might be publication bias (Begg’s test: P=0.300, Egger’s test: P=0.026).

Sensitivity Analysis To prove the reliability, we performed a sensitivity analysis in Table 4. Each study was individually excluded to ensure that each item met the objectives of the study. The value of SMD ranged from 0.38 to 0.87 in a sensitivity analysis comparing IL-10 levels in both DR and control groups by excluding one article at a time. The range of lower limits of 95%CI was from 0.11 to 0.34, and the upper limit was from 0.65 to 1.42. The F values remained significant heterogeneity. Therefore, this sensitivity analysis suggested that the results of the study are stable and reliable.

DISCUSSION

DR, the microvascular complication of diabetes, is a blinding eye disease that affects life quality in adults[33-35]. Studies have shown that the prevalence of DR in diabetic patients is about one-third or higher[36]. The number of patients
with type 2 diabetes is increasing in both developed and developing countries, which may lead to an increase in DR\(^{[37]}\). Inflammatory factors play an essential role in the progression of diabetes\(^{[38]}\). In addition to elevated levels of pro-inflammatory cytokines like IL-1 and IL-6, chemokines such as macrophage inflammatory protein-1β, interferon gamma-induced protein 1, and IL-8 were also elevated in PDR\(^{[39]}\). Meanwhile, it also produces anti-inflammatory cytokines such as IL-10 that regulate the inflammatory process. IL-10 is mainly produced by monocytes and has an inactivated property against macrophages\(^{[32,40]}\). IL-10 can inhibit antigen presentation by suppressing MHC-II expression in antigen-presenting cells\(^{[38]}\). At present, some literatures have been found to study the relationship between IL-10 and DR. Myśliwiec \textit{et al}\(^{[41]}\) suggested that in type 1 diabetes patients with different stages of DR, IL-10 levels allow for higher secretion activity and appear to be associated with protective functions for the progression of advanced diabetic complications. Suzuki \textit{et al}\(^{[24]}\) and Takeuchi \textit{et al}\(^{[25]}\) found that significantly elevated levels of IL-10 were detected in the vitreous of DR patients, but those studies did not analyze plasma samples. On the other hand, however, Wang \textit{et al}\(^{[38]}\) discovered that IL-10 was suppressed in peripheral B cells of patients with DR. Chernykh \textit{et al}\(^{[22]}\) did not found any significant change in the concentration of IL-10 in patients with DR. Therefore, further studies about the functions and mechanisms of IL-10 in DR are needed.

This Meta-analysis included 11 literature with 987 participants indicating that the IL-10 levels were higher in the DR group than in the control group. To better explain these results, we conducted subgroup analyses. First of all, we performed subgroup analyses according to sample sources. We recognized that the vitreous-derived samples and the aqueous humor-derived sample showed a significant increase in IL-10 concentration, while its concentrations in serum and plasma did not change significantly. As Figure 2 shows, in Takeuchi \textit{et al}’s study\(^{[25]}\), IL-10 was not detected from all of the vitreous samples in the control group, the data was not included in the

\[
\text{Table 4 Sensitivity analysis}
\]

<table>
<thead>
<tr>
<th>Study</th>
<th>SMD (95%CI)</th>
<th>Heterogeneity</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromberg-White \textit{et al}, 2013</td>
<td>0.75 (0.20, 1.30)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Chernykh \textit{et al}, 2015</td>
<td>0.85 (0.30, 1.40)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Kaviarasan \textit{et al}, 2015-serum</td>
<td>0.84 (0.28, 1.41)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Kaviarasan \textit{et al}, 2015-vitreous</td>
<td>0.74 (0.20, 1.28)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Koskela \textit{et al}, 2013-plasma</td>
<td>0.87 (0.34, 1.40)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Koskela \textit{et al}, 2013-vitreous</td>
<td>0.76 (0.22, 1.31)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Lee \textit{et al}, 2008</td>
<td>0.83 (0.27, 1.39)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Ozturk \textit{et al}, 2009</td>
<td>0.84 (0.26, 1.42)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Suzuki \textit{et al}, 2011</td>
<td>0.81 (0.25, 1.37)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Takeuchi \textit{et al}, 2015</td>
<td>0.77 (0.25, 1.28)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Wu \textit{et al}, 2017</td>
<td>0.75 (0.21, 1.30)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Xu \textit{et al}, 2015</td>
<td>0.38 (0.11, 0.65)</td>
<td>(P=0.0002)</td>
<td>69</td>
</tr>
<tr>
<td>Yabanoğlu \textit{et al}, 2014-serum</td>
<td>0.82 (0.29, 1.36)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
<tr>
<td>Yabanoğlu \textit{et al}, 2014-vitreous</td>
<td>0.80 (0.26, 1.34)</td>
<td>(P&lt;0.00001)</td>
<td>92</td>
</tr>
</tbody>
</table>

\[
\text{Figure 4 Subgroup analysis of IL-10 level according to DR type.}
\]

\[
\text{Figure 5 Assessment of publication bias. A funnel plot analysis of all included samples.}
\]
calculated results of Meta-analysis. However, the trend of that study also supported the increase of IL-10 in vitreous from patients with DR. Wu et al.\(^\text{[30]}\) found that the concentration of IL-10 gradually increases as the degree of DR increases. Thus, we performed a subgroup analysis based on the types of DR, and the outcomes showed a significant difference between the PDR group and the NPDR group.

Nevertheless, some limitations still remain in this Meta-analysis. For example, the majority of the control samples were collected from the hospital, thus selection bias could not be avoided. Besides, the number of included studies is not large enough. Moreover, significant heterogeneity and publication bias may be existed, while sensitivity analysis showed that sequentially omitting one study did not change the conclusion of the Meta-analysis. Despite these shortcomings, we still believe that the Meta-analysis could provide valuable evidence for the clinical significance of IL-10 in patients with DR.

According to the Meta-analysis, the concentration of IL-10 was significantly higher in the samples of DR group compared to the control group, particularly in vitreous samples. Further investigations are needed to reveal the roles and mechanisms of IL-10 in DR pathogenesis.

**ACKNOWLEDGEMENTS**

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**Conflicts of Interest:** Tan W, None; Zou JL, None; Yoshida S, None; Jiang B, None; Zhou YD, None.

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