Lack of Association between Serum Vitamin B₆, Vitamin B₁₂, and Vitamin D Levels with Different Types of Glaucoma: A Systematic Review and Meta-Analysis

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Abstract: Although vitamins play a major role in health, and their deficiency may be linked to symptoms of optic-nerve dysfunction, the association between serum vitamin levels and glaucoma in humans remains controversial. In this study, articles in the PubMed, Web of Science, and EMBASE databases were searched up to 25 March 2017. Nine studies on primary open-angle glaucoma (POAG), four studies on normal tension glaucoma (NTG), and six studies on exfoliative glaucoma (EXG) were retrieved. The combined results showed no differences in the levels of serum vitamin B₆ between POAG (p = 0.406) and EXG (p = 0.139) patients and controls. The weighted mean differences (WMDs) with 95% confidence intervals (CIs) were 2.792 ng/mL (−3.793 to 9.377) and 1.342 ng/mL (−3.120 to 0.436), respectively. There was no difference between POAG (p = 0.952), NTG (p = 0.757), or EXG (p = 0.064) patients and controls in terms of serum vitamin B₁₂. The WMDs with 95% CIs were 0.933 pg/mL (−31.116 to 29.249), 6.652 pg/mL (−35.473 to 48.777), and 49.946 pg/mL (−102.892 to 3.001), respectively. The serum vitamin D levels exhibited no differences (p = 0.064) between POAG patients and controls; the WMD with 95% CI was 2.488 ng/mL (−5.120 to 0.145). In conclusion, there was no association found between serum vitamin B₆, vitamin B₁₂, or vitamin D levels and the different types of glaucoma.

Keywords: glaucoma; vitamin B₆; vitamin B₁₂; vitamin D; meta-analysis

1. Introduction

Glaucoma is the second leading cause of irreversible blindness worldwide; its prevalence is expected to increase from 60.5 million people in 2010 to 79.6 million by 2020 [1]. The common characteristic features of glaucoma are retinal ganglion cell apoptosis and visual field changes [2]. There are two types of adult-onset glaucoma, as follows: open-angle glaucoma (OAG), in which the angle of the anterior chamber is anatomically open, and angle-closure glaucoma, in which the angle is anatomically closed. OAG is the most common type of glaucoma; based on the intraocular pressure...
(IOP), it is divided into primary open-angle glaucoma (POAG; IOP > 21 mm Hg) and normal tension
glaucoma (NTG; untreated IOP ≤ 21 mm Hg). Exfoliative glaucoma (EXG) is characterized by high
IOP and worse 24-h IOP, and it represents the most common type of secondary OAG. Various risk
factors have been investigated to evaluate the associations with the development and progression of
glaucoma, including increased age, gender, high IOP, and genetic variants [3,4]. However, the precise
mechanisms involved in glaucoma are yet to be determined.

Recently, Williams et al. [5] reported that vitamin B₃ can modulate mitochondrial vulnerability
and prevent glaucoma in mice. Although many publications have reported a difference in serum
vitamin levels between patients with glaucoma and normal subjects, the association between serum
vitamin levels and glaucoma in humans remains controversial. Turgut et al. [6] showed that the serum
vitamin B₆ levels are significantly higher in POAG and NTG patients than in controls, but others
reported no significant difference in serum vitamin B₆ levels among these groups [7–9]. Several studies
have shown that serum vitamin B₁₂ levels are elevated in NTG, POAG, and EXG [6,10,11], but others
have reported decreased levels of serum vitamin B₁₂ [9,12]. Moreover, although most studies have
found that the serum vitamin D level is decreased in glaucoma patients compared with controls [13,14],
the difference in vitamin D levels between glaucoma patients and controls is limited.

Vitamins are dietary components that are required for the proper function of the methylation cycle,
monoamine oxidase production, DNA synthesis, and phospholipid repair and maintenance [15,16].
They play a major role in health, and their deficiencies may be linked to symptoms of neuronal
dysfunction. However, the number of published papers evaluating the associations between serum
vitamin (A, C, E) levels and different types of glaucoma is relatively limited. Therefore, we aim to
perform a systematic review and meta-analysis by combining individual studies and summarizing
an overall effect size for the association between vitamin B₆, vitamin B₁₂, and vitamin D levels with
different types of glaucoma.

2. Methods

2.1. Publication Search

Eligible articles were aggregated from three databases, namely PubMed, Web of Science, and
EMBASE; these were published in the English language from 1 January 1990 to 25 March 2017.
The following search terms were used: “glaucoma” (in the title) and “vitamin” (in the title/abstract).
Moreover, a manual search was performed by checking the reference lists of the reports on clinical
trials, meta-analyses, and systematic reviews that were examined. Two reviewers (Shengjie Li and
Danhui Li) completed the literature search independently.

2.2. Inclusion Criteria

All studies had to meet the following inclusion criteria:

1. The investigation involved random sampling or cluster sampling;
2. Two or more comparison groups (glaucoma and control groups) were included;
3. Healthy subjects were recruited for the control group;
4. A laboratory assessment of serum or plasma vitamin levels (vitamin B₆/vitamin B₁₂/vitamin D)
was conducted;
5. The study was published in English;
6. The full text of the article was accessible; and
7. The subjects were human.

2.3. Study Selection and Data Extraction

Study selection was performed by two independent investigators (Shengjie Li and Danhui Li)
according to the inclusion criteria listed. From each study, the following data were collected and
reviewed independently by the two investigators (Shengjie Li and Danhui Li): the first author’s name, country/region, publication year, mean age of participants, sample size, type of glaucoma, and considered vitamin (vitamin B\textsubscript{6}/vitamin B\textsubscript{12}/vitamin D). Moreover, we conducted a focused discussion to resolve any disagreements.

2.4. Quality Assessment

The quality assessments for the study were based on an examination of the previously reported guidelines for glaucoma studies [17,18]. We developed a quality score for each included study that was reviewed independently by two investigators (Shengjie Li and Danhui Li) evaluating six items, as follows:

1. Was the study design clearly described?
2. Were the diagnostic criteria and clinical examinations comprehensive and standardized?
3. Were the participant selection procedures reported clearly?
4. Was the participant enrollment duration provided?
5. Were the age and sex of eligible participants clearly described?
6. Were the serum vitamin B\textsubscript{6}/B\textsubscript{12}/D measurement methods clearly reported?

In the scoring system, for each quality item, a response of “clear or adequate” resulted in a score of 1 point, whereas a response of “no” received a score of 0 points. The study was considered as being of adequate quality if the quality score was greater than or equal to 4. Studies of inadequate quality were excluded from this meta-analysis.

2.5. Statistical Analysis

The weighted mean differences (WMDs) in vitamin B\textsubscript{6}, vitamin B\textsubscript{12}, and vitamin D levels between glaucoma (POAG/NTG/EXG) and control groups and 95% confidence intervals (CIs) were calculated for each study. The heterogeneity of the pooled studies was estimated using the \(\chi^2\)-based Q statistic and \(I^2\) metrics. A random-effects model was used if heterogeneity was observed (\(p < 0.05\) or \(I^2 > 60\%\)); otherwise, a fixed-effects model was applied. A funnel plot analysis and Egger’s test were performed to assess potential publication bias. We performed a sensitivity analysis to evaluate the stability of the results through the leave-one-out strategy. This method uses the sequential omission of individual studies in every comparison to determine whether there is a significant alteration of the combined values. A value of \(p < 0.05\) was considered statistically significant. The statistical analyses were performed using Comprehensive Meta-Analysis version 2.0 (Biostat, Englewood Cliffs, NJ, USA).

3. Results

3.1. Search Results and Study Characteristics

A flowchart illustrating the article search process is presented in Figure 1. The initial search strategy identified 72 studies in PubMed, 69 in Web of Science, and 40 in EMBASE. From the 181 studies, 168 were excluded. Finally, 13 studies [6–14,19–22] were included in this meta-analysis. The detailed characteristics of each included study are presented in Table 1. The quality scores of these studies ranged from 4 to 6, with a mean of 5.08. Detailed scoring results are presented in Table 2.

According to the type of glaucoma, 13 studies were categorized into three groups. Since some studies discussed different types of glaucoma, they could be included in more than one group. Thus, there were nine studies included in the POAG group [6–8,10–14,19], four in the NTG group [6,9,10,12], and six in the EXG group [6,11,12,20–22]. Moreover, according to the different types of vitamins considered (vitamin B\textsubscript{6}/vitamin B\textsubscript{12}/vitamin D), the following categorizations were identified:
(1) The POAG group: Three studies considered vitamin B\textsubscript{6} (109 cases and 115 controls), six considered vitamin B\textsubscript{12} (222 cases and 249 controls), and three considered vitamin D (513 cases and 5629 controls);

(2) The NTG group: Two studies considered vitamin B\textsubscript{6} (90 cases and 82 controls), four considered vitamin B\textsubscript{12} (123 cases and 176 controls), and 0 considered vitamin D; and

(3) The EXG group: Three studies considered vitamin B\textsubscript{6} (144 cases and 146 controls), six considered vitamin B\textsubscript{12} (228 cases and 240 controls), and one considered vitamin D (70 cases and 70 controls).

Figure 1. Study selection flow chart.

3.2. Meta-Analysis of the Association of Vitamin B\textsubscript{6} with POAG and EXG

The combined results showed no difference in the serum vitamin B\textsubscript{6} levels between POAG patients and controls ($p = 0.406$; Figure 2A). The WMD was 2.792 ng/mL (95% CI = −3.793 to 9.377), and there was significant between-study heterogeneity for vitamin B\textsubscript{6} among the available studies ($p < 0.0001$, $I^2 = 89.581\%$). Moreover, there was no significant difference in serum vitamin B\textsubscript{6} between EXG patients and controls ($p = 0.139$; Figure 2B). The WMD was 1.342 ng/mL (95% CI = −3.120 to 0.436), and there was no significant heterogeneity for vitamin B\textsubscript{6} between the available studies ($p = 0.082$, $I^2 = 59.982\%$).
Table 1. Characteristics of Studies of Vitamin B<sub>6</sub>, Vitamin B<sub>12</sub>, and Vitamin D with Different Types of Glaucoma.

| First Author          | Year | Country     | Glaucoma Group | Control Group |                     |                     |
|-----------------------|------|-------------|----------------|---------------|---------------------|---------------------|
|                       |      |             | No. Age Vitamin B<sub>6</sub> ng/mL | Vitamin B<sub>12</sub> pg/mL | Vitamin D ng/mL No. Age Vitamin B<sub>6</sub> ng/mL | Vitamin B<sub>12</sub> pg/mL | Vitamin D ng/mL |
| **POAG**              |      |             |                 |               |                     |                     |
| Lv et al. [13]        | 2016 | China       | 73 61.03 ± 2.75 | 26.37 ± 5.83 | 71 60.14 ± 3.03 | 30.43 ± 3.91       |
| López-Riquelme et al. [10] | 2015 | Spain       | 48 50.0 ± 9.4  | 404.2 ± 198.2 | 75 43.7 ± 12.4    | 425.7 ± 137.7       |
| Roedl et al. [8]      | 2007 | Germany     | 39 69.3 ± 8.4  | 12.64 ± 6.50 | 39 70.5 ± 10.7    | 13.46 ± 9.00        |
| Roedl et al. [7]      | 2008 | Germany     | 36 67.3 ± 8.2  | 12.50 ± 7.15 | 36 68.5 ± 9.8     | 13.12 ± 7.61        |
| Yoo et al. [19]       | 2014 | Korea       | 290 63.3 ± 10.7| 21.05 ± 12.61| 164 75.1 ± 8.5    | 18.7 ± 6.6          |
| Goncalves et al. [14] | 2015 | France      | 150 73.0 ± 7.9 | 232.84 ± 67.55| 19 55.63 ± 4.04   | 261.84 ± 126.22     |
| Cumurcu et al. [12]   | 2006 | Turkey      | 25 56.76 ± 12.58| 232.84 ± 67.55| 19 55.63 ± 4.04   | 261.84 ± 126.22     |
|                       | 2011 | Italy       | 40 68.71 ± 8.65| 444.9 ± 167.17| 40 69.23 ± 7.21   | 397.15 ± 118.68     |
| Turgut et al. [6]     | 2010 | Turkey      | 34 58 ± 7.5    | 30.22 ± 12.15 | 40 62 ± 8.1       | 20.09 ± 5.54        |
| **NTG**               |      |             |                 |               |                     |                     |
| López-Riquelme et al. [10] | 2015 | Spain       | 15 45.3 ± 12.1 | 471.7 ± 177.6 | 75 43.7 ± 12.4    | 425.7 ± 137.7       |
| Cumurcu et al. [12]   | 2006 | Turkey      | 18 57.77 ± 7.27| 262.33 ± 85.94| 19 55.63 ± 4.04   | 261.84 ± 126.22     |
| Rössler et al. [9]    | 2010 | Germany     | 42 65.5 ± 12.1 | 14.45 ± 12.89 | 42 63.1 ± 11.5    | 13.57 ± 10.41       |
| Turgut et al. [6]     | 2010 | Turkey      | 48 56 ± 9.5    | 30.50 ± 11.29 | 40 62 ± 8.1       | 20.09 ± 5.54        |
| **EXG**               |      |             |                 |               |                     |                     |
| Puustjärvi et al. [20]| 2004 | Finland     | 36 77.4 ± 6.0  | 33.3 ± 20.1   | 36 77.2 ± 5.4     | 37.9 ± 28.2         |
| Roedl et al. [22]     | 2007 | Germany     | 70 70.3 ± 8.2  | 10.29 ± 5.73  | 70 68.4 ± 11.6    | 12.54 ± 6.40        |
| Cumurcu et al. [12]   | 2006 | Turkey      | 24 61.66 ± 10.05| 209.37 ± 104.44| 19 55.63 ± 4.04   | 261.84 ± 126.22     |
| Turkcu et al. [21]    | 2013 | Turkey      | 24 67.0 ± 6.9  | 232.2 ± 104.8 | 35 69.6 ± 6.5     | 372.8 ± 138.8       |
| Tranchina et al. [11] | 2011 | Italy       | 36 69.58 ± 5.92| 434.53 ± 141.46| 40 69.23 ± 7.21   | 397.15 ± 118.68     |
| Turgut et al. [6]     | 2010 | Turkey      | 38 63 ± 6.3    | 22.81 ± 11.71 | 40 62 ± 8.1       | 20.09 ± 5.54        |
Table 2. Quality Scores of Individual Studies.

| First Author                  | (1) | (2) | (3) | (4) | (5) | (6) | Total |
|-------------------------------|-----|-----|-----|-----|-----|-----|-------|
| Lv et al. [13]                | 1   | 1   | 1   | 1   | 1   | 1   | 6     |
| López-Riquelme et al. [10]    | 1   | 1   | 1   | 0   | 1   | 1   | 5     |
| Roedl et al. [8]              | 1   | 0   | 0   | 1   | 1   | 1   | 4     |
| Roedl et al. [7]              | 1   | 0   | 1   | 1   | 1   | 1   | 5     |
| Yoo et al. [19]               | 1   | 1   | 1   | 1   | 1   | 1   | 5     |
| Goncalves et al. [14]         | 1   | 1   | 1   | 1   | 1   | 1   | 5     |
| Cumurcu et al. [12]           | 1   | 1   | 0   | 1   | 1   | 1   | 5     |
| Tranchina et al. [11]         | 1   | 0   | 1   | 1   | 1   | 1   | 5     |
| Turgut et al. [6]             | 1   | 1   | 0   | 0   | 1   | 1   | 4     |
| Rossler et al. [9]            | 1   | 1   | 1   | 0   | 1   | 1   | 4     |
| Roedl et al. [22]             | 1   | 1   | 0   | 0   | 1   | 1   | 4     |
| Turkcu et al. [21]            | 1   | 1   | 1   | 0   | 1   | 1   | 5     |
| Puustjryvi et al. [20]        | 1   | 1   | 1   | 1   | 1   | 1   | 6     |

Note: (1) Was study design clearly described? (2) Were diagnostic criteria and clinical examinations comprehensive and standardized? (3) Were participant selection procedures reported clearly? (4) Was participant enrollment duration provided? (5) Were the age and sex of eligible participants clearly described? (6) Were vitamin B<sub>6</sub>/B<sub>12</sub>/D measurement methods clearly reported?

Figure 2. Forest plot of random effects meta-analysis showing the association of serum vitamin B<sub>6</sub> levels with POAG (A) and EXG (B).

3.3. Meta-Analysis of the Association of Vitamin B<sub>12</sub> with POAG, NTG, and EXG

In the combined results, the levels of serum vitamin B<sub>12</sub> exhibited no difference between POAG patients and controls ($p = 0.952$; Figure 3A). The WMD was 0.933 pg/mL (95% CI = −31.116 to 29.249), and there was no significant between-study heterogeneity for vitamin B<sub>12</sub> among the available studies ($p = 0.555, I^2 = 0.000%$).
The level of serum vitamin B\textsubscript{12} showed no significant difference between NTG patients and controls ($p = 0.757$; Figure 3B). The WMD was 6.652 pg/mL (95% CI = −35.473 to 48.777), and there was no significant between-study heterogeneity for vitamin B\textsubscript{12} among the available studies ($p = 0.653$, $I^2 = 0.000\%$). Moreover, there was no significant difference in serum vitamin B\textsubscript{12} between EXG patients and controls ($p = 0.064$; Figure 3C). The WMD was 49.946 pg/mL (95% CI = −102.892 to 3.001), and there was significant between-study heterogeneity for vitamin B\textsubscript{12} among the available studies ($p < 0.001$, $I^2 = 78.935\%$).

![Figure 3. Forest plot of random effects meta-analysis showing the association of serum vitamin B\textsubscript{12} levels with POAG (A), NTG (B), and EXG (C).](image)

### 3.3. Meta-Analysis of the Association between Vitamin D and POAG

The combined results showed that there was no significant difference in serum vitamin D levels between POAG patients and controls ($p = 0.064$; Figure 4). The WMD was 2.488 ng/mL (95% CI = −5.120 to 0.145), and there was significant between-study heterogeneity for vitamin D among the available studies ($p < 0.001$, $I^2 = 87.229\%$).
Moreover, one study influenced the meta-analysis results regarding the association between vitamin D and POAG (Table 3). Moreover, one study influenced the meta-analysis results regarding the association between vitamin B₁₂ and EXG (Table 3), and one study influenced the results regarding the association between vitamin B₆ and EXG (Table 3), two studies influenced the results regarding the association between vitamin B₁₂ and EXG (Table 3), and one study influenced the results regarding the association between vitamin B₁₂ and EXG (Table 3), and one study influenced the results regarding the association between vitamin B₁₂ and EXG (Table 3).

### 3.4. Analysis of Publication Bias

There were no obvious asymmetries in the funnel plots. The p-values exceeded 0.05 for the following groups: vitamin B₆ in POAG ($t = 3.369, \ p = 0.07, \ 95\% \ CI = -13.19 \ to \ 69.22; \ Figure \ 5A$), vitamin B₆ in EXG ($t = 0.271, \ p = 0.83, \ 95\% \ CI = -29.92 \ to \ 31.22; \ Figure \ 5B$), vitamin B₁₂ in POAG ($t = 0.463, \ p = 0.67, \ 95\% \ CI = -3.43 \ to \ 4.80; \ Figure \ 5C$), vitamin B₁₂ in NTG ($t = 0.732, \ p = 0.54, \ 95\% \ CI = -13.44 \ to \ 9.53; \ Figure \ 5D$), vitamin B₁₂ in EXG ($t = 0.271, \ p = 0.83, \ 95\% \ CI = -29.92 \ to \ 31.22; \ Figure \ 5E$), and vitamin D in POAG ($t = 1.332, \ p = 0.41, \ 95\% \ CI = -40.72 \ to \ 33.00; \ Figure \ 5F$).

### 3.5. Sensitivity Analysis

In the meta-analysis of the association between serum vitamin levels and the different types of glaucoma (POAG/NTG/EXG), the sensitivity analysis revealed that one study had a slight influence on the result; the results from the sensitivity analysis showed no associations of serum vitamin B₆ levels with POAG, vitamin B₁₂ with POAG, or vitamin B₁₂ with NTG after this study was excluded (Table 3). Moreover, one study influenced the meta-analysis results regarding the association between vitamin B₆ and EXG (Table 3), two studies influenced the results regarding the association between vitamin B₁₂ and EXG (Table 3), and one study influenced the results regarding the association between vitamin D and POAG (Table 3).

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**Figure 4.** Forest plot of random effects meta-analysis showing the association between serum vitamin D and POAG.

**Figure 5.** Cont.
3.5. Analysis of Publication Bias

There were no obvious asymmetries in the funnel plots. The p-values exceeded 0.05 for the following groups: vitamin B6 in POAG (t = 3.369, p = 0.07, 95% CI = −13.19 to 69.22; Figure 5A), vitamin B6 in EXG (t = 0.271, p = 0.83, 95% CI = −29.92 to 31.22; Figure 5B), vitamin B12 in POAG (t = 0.463, p = 0.67, 95% CI = −3.43 to 4.80; Figure 5C), vitamin B12 in NTG (t = 0.732, p = 0.54, 95% CI = −13.44 to 9.53; Figure 5D), vitamin B12 in EXG (t = 0.271, p = 0.83, 95% CI = −29.92 to 31.22; Figure 5E), and vitamin D in POAG (t = 1.332, p = 0.41, 95% CI = −40.72 to 33.00; Figure 5F).

3.6. Sensitivity Analysis

**Table 3. Sensitivity Analysis Using the Leave-one-out Strategy.**

| Study Omitted          | Point   | 95% CI      | p-Value |
|------------------------|---------|-------------|---------|
| POAG (vitamin B6)      |         |             |         |
| Roedl et al., 2007 [8] | 4.683   | −5.851−15.216| 0.384   |
| Roedl et al., 2008 [7] | 4.590   | −6.140−15.320| 0.402   |
| Turgut et al., 2010 [6]| 1.244   | −3.155−1.720 | 0.564   |
| EXG (vitamin B6)       |         |             |         |
| Puustjrvii et al., 2004 [20] | −0.130  | −0.397−0.138 | 0.341  |
| Roedl et al., 2007 [22] | 0.065   | −0.257−0.386 | 0.693   |
| Turgut et al., 2010 [6] | −0.308  | −0.579−0.037 | 0.026 * |
| POAG (vitamin B12)     |         |             |         |
| López-Riquelme et al., 2015 [10] | 0.056  | −0.155−0.267 | 0.602  |
| Roedl et al., 2007 [8] | 0.024   | −0.176−0.224 | 0.811   |
| Roedl et al., 2008 [7] | −0.001  | −0.199−0.198 | 0.995   |
| Cumurcu et al., 2006 [12] | 0.040  | −0.151−0.232 | 0.682   |
| Tranchina et al., 2011 [11] | −0.057  | −0.258−0.143 | 0.575   |
| Turgut et al., 2010 [6] | −0.008  | −0.207−0.191 | 0.936   |
| NTG (vitamin B12)      |         |             |         |
| López-Riquelme et al., 2015 [10] | −0.061  | −0.333−0.211 | 0.661   |
| Cumurcu et al., 2006 [12] | 0.013   | −0.250−0.277 | 0.921   |
| Rossler et al., 2010 [9] | 0.089   | −0.208−0.387 | 0.556   |
| Turgut et al., 2010 [6] | 0.021   | −0.280−0.321 | 0.892   |
Table 3. Cont.

| Study Omitted | Point   | 95% CI              | p-Value |
|---------------|---------|---------------------|---------|
| EXG (vitamin B<sub>12</sub>) |         |                     |         |
| Puustjrvi et al., 2004 [20] | −62.354 | −121.997−2.711 | 0.040<sup>Δ</sup> |
| Roedl et al., 2007 [22]   | −41.399 | −102.731−19.333 | 0.186   |
| Cumurcu et al., 2006 [12] | −49.770 | −112.833−13.294 | 0.122   |
| Turkcu et al., 2013 [21]  | −32.109 | −81.451−17.234 | 0.202   |
| Tranchina et al., 2011 [11] | −67.396 | −118.976−15.815 | 0.010<sup>Δ</sup> |
| Turgut et al., 2010 [6]   | −46.934 | −108.324−14.455 | 0.134   |
| POAG (vitamin D)          |         |                     |         |
| Lv et al., 2016 [13]      | −1.468  | −3.865−0.928        | 0.230   |
| Yoo et al., 2014 [19]     | −3.864  | −5.295−2.433        | <0.0001<sup>Ω</sup> |
| Goncalves et al., 2015 [14] | −2.253  | −5.641−1.134        | 0.192   |

Note: CI = confidence interval; Δ = influenced the meta-analysis results regarding the association between vitamin B<sub>12</sub> and EXG; * = influenced the meta-analysis results regarding the association between vitamin B<sub>6</sub> and EXG; Ω = influenced the meta-analysis results regarding the association between vitamin D and POAG.

4. Discussion

The relationship between serum vitamin levels and the presentation of NTG, POAG, or EXG remains uncertain; however, serum vitamin levels are considered to be associated with NTG, POAG, and EXG in terms of protecting neuronal function [23]. Thus, we performed the present meta-analysis to clarify this relationship. However, the meta-analysis results suggested that there is no evidence to confirm the association of serum vitamin levels with different types of glaucoma.

The exact biological mechanisms of the action of serum vitamins (vitamin B<sub>6</sub>/vitamin B<sub>12</sub>/vitamin D) in NTG, POAG, and EXG are not fully understood. Salari et al. [24] found that adding vitamin D to routine disease therapy had no significant effect on the thickness of the retinal nerve fiber layer or macula in patients with optic neuritis in a randomized, placebo-controlled trial study. In contrast, a recent article proposed that 1α,25-Dihydroxyvitamin D(3), or an analog thereof, may be used to treat glaucoma [25]. Romano et al. [26] suggested that vitamin B<sub>12</sub> treatment represents a powerful strategy to accelerate not only re-epithelization, but also corneal re-innervation after mechanical injury. Contradictory results have also been reported for the association between serum vitamin B<sub>12</sub> and glaucoma [6,9–12].

4.1. Vitamin B<sub>6</sub> in POAG and EXG

Some researchers have suggested that vitamin B<sub>6</sub> is associated with POAG and EXG. Turgut et al. [6] performed a case-control study and reported that the serum vitamin B<sub>6</sub> levels were significantly increased in POAG and NTG patients. However, contrasting results, where in the vitamin B<sub>6</sub> levels were not significantly different between POAG or EXG and controls, were also reported by Roedl et al. [7,8] and Rössler et al. [9]. In this subgroup meta-analysis, we did not detect any difference in serum vitamin B<sub>6</sub> between POAG or EXG patients and controls. However, there was significant heterogeneity in POAG research concerning vitamin B<sub>6</sub> among the available studies; such heterogeneity was not evident among EXG studies.

The significant heterogeneity concerning POAG research may be partially explained in terms of variances in nutritional status, diet, and lifestyle in the different populations studied (German vs. Turkish), as well as the vitamin B<sub>6</sub> detection kits that were used (Bio-Rad, Munich, Germany vs. Shimadzu Corporation, Kyoto, Japan). In addition, the sensitivity analysis concerning serum vitamin B<sub>6</sub> levels in patients with POAG indicated that the result was not greatly influenced by the exclusion of any individual study. However, the sensitivity analysis also showed that one study [6] influenced the meta-analysis results regarding the association between vitamin B<sub>6</sub> and EXG.
4.2. Vitamin B\textsubscript{12} in POAG, NTG, and EXG

Some studies have claimed that vitamin B\textsubscript{12} is associated with POAG, NTG, and EXG. Turgut et al. [6], López-Riquelme et al. [10], and Tranchina et al. [11] suggested that glaucoma patients have higher serum vitamin B\textsubscript{12} levels than normal controls. In contrast, two publications claimed that serum vitamin B\textsubscript{12} levels were lower in individuals with glaucoma than in healthy controls [9,12]. The combined results from this meta-analysis showed that there was no difference in the levels of serum vitamin B\textsubscript{12} between POAG, NTG, or EXG patients and controls ($p = 0.952$). However, significant heterogeneity in EXG studies on vitamin B\textsubscript{12} was observed, and two studies influenced the meta-analysis results regarding the association between vitamin B\textsubscript{12} and EXG. This may be partially explained by the variances in sample number (24 to 70). The different definitions of EXG and the difference in the vitamin B\textsubscript{12} measurements may also have influenced the outcomes. At present, the most frequently used methods are a competitive chemiluminescent enzyme immunoassay [11,12,21], the time-resolved fluoroimmunoassay method [20], and an immunoassay [22]. However, these methods have not been standardized, so between-study comparisons are difficult. Furthermore, our sensitivity analysis indicated that the validity of the summary effect was stable in POAG and NTG studies, and this did not change materially when individual studies were excluded.

4.3. Vitamin D in POAG

No significant difference in serum vitamin D levels between POAG patients and controls was detected in this meta-analysis. However, there was significant between-study heterogeneity among the available studies on vitamin D, and one study influenced the meta-analysis results regarding the association between vitamin D and POAG. The reason for this maybe that one included study was a cross-sectional study, while the other two were case-control studies. Moreover, significant differences in the sample size and vitamin D measurements (enzyme-linked immunosorbent assay vs. radioimmunoassay) were detected, which may have also influenced the outcome. A randomized trial with vitamin D supplementation may be more valuable to evaluate the temporal and causal relationship between vitamin D and glaucoma risk.

Although a standard search strategy and a thorough computerized search method were applied, certain limitations of our meta-analysis should be considered. First, the studies differed widely in terms of the study populations’ characteristics and measurement techniques. Second, although the quality scores of the studies ranged from 4 to 6, representing high quality data, the studies included in this meta-analysis were often small-scale, single-center studies.

5. Conclusions

In this meta-analysis, we reported that there is no association between serum vitamin B\textsubscript{6}, vitamin B\textsubscript{12}, and vitamin D levels with different types of glaucoma. However, it seems a little early to draw a conclusion based on the limited number of available studies so far. Consequently, a focus on the possible role of vitamins in the pathogenesis of different types of glaucoma may be highly desirable in future research. Therefore, a forward-looking, multi-center study with a larger sample size ought to be conducted.

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