**Association of Severity of Primary Open-Angle Glaucoma with Serum Vitamin D Levels**

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**Abstract**

**Background:** The relationship between primary open-angle glaucoma (POAG), a leading cause of permanent vision loss, and serum vitamin D levels is still the subject of ongoing research. **This study aimed to** analyze the connection between serum vitamin D levels and the severity of POAG. **Methods:** This case-control study was completed on 25 POAG patients and 25 control subjects. Based on changes in the MD of the visual field, the group of POAG patients was divided into mild, moderate, advanced, and severe groups. Every participant underwent a thorough history taking and an examination of their eyes. An enzyme-linked immunosorbent test was employed in order to establish serum vitamin D levels (ELISA). **Results**: The mean serum vitamin D level in the POAG group was 27.16 ng/mL (± 11.53), while that in the control group was 49.62 ng/mL (± 11.42). ROC analysis demonstrated that vitamin D was an excellent discriminator between the two groups (AUC = 0.926, 95% CI: 0.844–1.000). Vitamin D can predict severe POAG (AUC = 0.947, 95% CI: 0.858–1.000) with a cut-off value of ≤16.32 ng/mL. Logistic regression analysis indicated that reduced vitamin D levels were a highly significant POAG predictor. Ordinal regression analysis showed that, in multivariable analysis, only vitamin D levels were a predictor for greater grades of POAG severity. **Conclusion:**The current study found a high correlation between serum vitamin D levels and illness severity and that vitamin D deficiency was a reliable predictor of POAG occurrence.

**Keywords:** Primary open-angle glaucoma; Vitamin D; Severity; Prediction.

**Introduction**

The most typical reason for permanent blindness is glaucoma. Progressive optic neuropathy is one way to describe the condition when blindness develops as a result of progressive loss of the visual field. Often identified only after irreparable loss of vision, Of the various types of glaucoma, primary open-angle glaucoma (POAG) holds the position of the most prevalent. Early on, this condition usually manifests itself without causing any noticeable symptoms (1). Given the chronic nature of these irreversible processes, medical professionals are working to identify the risk factors that contribute to the progression of glaucoma. Increased intraocular pressure (IOP), advanced age, and favorable family history are recognized risk factors. It has been noted that black communities possess a greater frequency of glaucoma than white and mixed-race groups. In a similar vein, black individuals had a higher mean IOP than mixed-race or white patients (2).

Studies suggest a connection between glaucoma and serum 25-OH vitamin D levels. It was discovered that low 25-OH vitamin D levels in the serum increase the risk of developing open-angle glaucoma (OAG) (3-5).

Vitamin D impacts other systems in addition to bone metabolism. Research conducted in the past few decades has shown the multifunctionality of vitamin D as a chemical and its importance to health. Depression, autoimmune disorders, cancer, diabetes, osteoporosis, and cardiovascular disease are all linked to vitamin D insufficiency. In addition, vitamin D affects gene regulation, angiogenesis, apoptosis, proliferation, and cell differentiation. Previous research has also demonstrated correlations between vitamin D levels and a number of different ocular disorders (6).

In a group of elderly Caucasians, serum 25-OH vitamin D insufficiency was linked to a smaller ganglion cell complex. Furthermore, among young adults in Australia, reduced 25-OH vitamin D levels in the serum were linked to a higher incidence of myopia. Furthermore, a lack of vitamin D in elderly persons was linked to a lower level of total visual acuity (4, 7).

Vitamin D receptor activation or indirect modulation of calcium homeostasis are two of the protective activities of vitamin D that may impact potential processes for the development of glaucoma. Research has indicated that a primary factor contributing to neurodegenerative damage of optic nerve axons and ganglion cell bodies is an immune system imbalance. Additionally, by opening calcium channels, vitamin D controls oxidative stress in neurons, which is a major contributor to glaucomatous optic nerve damage (8).

A potential reason for the association between low vitamin D levels and glaucoma could be associated with the diminished blood supply to the eyes. Yang claims that vitamin D improves vasodilation, is dependent on endothelial cells, has an effect on microvessel and peripheral circulation, and controls the renin-angiotensin system. By increasing ocular blood flow, inhibition of the renin-angiotensin system has been demonstrated in an animal-based study to reduce the incidence of glaucoma (9, 10). This study set out to ascertain the association between serum vitamin D levels and POAG severity.

**Patients and Methods**

**Patients:**

This case-control research was completed at Benha University Hospital's ophthalmology department on 50 volunteers. The study was conducted from April 2022 to March 2023, spanning a full year.

There were 25 POAG patients and 25 control participants in the trial. Patients had to have glaucomatous visual field damage, an open angle as assessed by a Gonio lens examination, high intraocular pressure, distinctive symptoms of optic disc injury, and no secondary glaucoma signs in order to be categorized as having POAG. Individuals in the control group did not exhibit any clinical indications of glaucomatous optic neuropathy, had an intraocular pressure of ≤ 21 mmHg, and had no family history of first-degree relatives developing POAG. Based on baseline visual field (VF) loss, POAG patients were categorized into four severity groups: mean deviation [MD] values are classified as mild (less than -6 dB), moderate (ranging from -6.01 to -12 dB), severe (ranging from -12.01 to -20 dB), and advanced (MD between -12.01 and -20 dB). This was done to aid in reporting (MD worse than -20.01 dB). Serum vitamin D levels were measured for each subject, and their association with the degree of POAG severity was recorded.

The patients gave their informed written consent. Each patient was given a code number and an explanation of the study's objectives. Once the Research Ethics Committee of Benha University Faculty of Medicine has given its permission, the study was carried out. **Inclusion criteria were** patients of either sex aged over 18 years with OAG.

**Exclusion criteria were** disorders or drugs that alter vitamin D levels (e.g., Chronic pancreatitis, celiac disease, Crohn's disease, hyperparathyroidism, renal failure), closed or narrow angles, secondary glaucoma, history of hepatitis C or HIV infection.

**Methods:**

All studied cases underwent the following: Detailed history taking, including age, sex, the length of the condition, the duration of the treatment and the daily dosage, detailed visual complaints, past ocular history of diseases and surgeries, other associated systemic diseases, and use of other medications.

**Ocular examination**

Ocular examinations involved examination of the pupil's response in conjunction with the following tests: center corneal thickness (CCT) assessment using anterior segment optical coherence tomography, best-corrected visual acuity (BCVA) using Snellen's chart testing, anterior segment assessment utilizing a slit lamp examination, and intraocular pressure measurement using an applanation tonometer (OPTOVUE, Inc., Fremont, CA), fundus examination using a +20 D lens to assess the retinal periphery and a +90 D lens for posterior pole biomicroscopy, mean deviation (MD) of visual field (VF) using Humphrey visual field testing, and gonioscopic examination using a 3-Mirror Zeiss Gonio lens.

**Serum 25-OH Vitamin D:**

An enzyme-linked immunosorbent assay (ELISA) kit was utilized to measure the amount of free serum 25-OH vitamin D (Future Diagnostics and DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium).

**Approval code:**

**Statistical analysis**

The Statistical Package for Social Science (IBM Corp., Released 2017) was utilized to process the data that were gathered. Armonk, NY: IBM Corp.; IBM SPSS Statistics for Windows, Version 25.0. For numerical data, descriptive statistics like mean, standard deviation, median, and range were utilized; for non-numerical data, frequency and percentage were used. Student T Test was used to contrast the means of two study groups; One Way ANOVA was utilized to analyze parametric variables for more than two study groups; Mann Whitney Test (U test) was employed to look into non-parametric variables between two groups; and Kruskal Wallis test was employed to investigate non-parametric variables for more than two study groups. Correlations between qualitative variables were explored utilizing Monte-Carlo and Chi-Square tests. The relationship between quantitative variables was evaluated by correlation analysis. The accuracy of diagnostic measures was indicated by AUC, while sensitivity and specificity were measured by the ROC Curve. For categorical dependent variables, risk factors were forecasted using logistic and ordinal regression analysis. The odds ratio (OR) was utilized to quantify the relationship between exposure and outcome. The precision of OR was evaluated with a 95 % CI, and statistical significance was established as p < 0.05.

**Results**

**General, clinical, and laboratory characteristics:**

No significant variations were observed between studied cases and controls concerning age (P = 0.061) and gender (P = 0.258). POAG group was significantly associated with lower VA in contrast to the control group (P < 0.001). The median visual acuity of the POAG group was 0.50, while that of the control group was 0.80. The mean±SD IOP of POAG patients was 18.80±3.46 mmHg, while that of the control group was 14.96±3.06 mmHg. [The mean±SD CCT of POAG patients was 533.1 ± 31.45 micrometers, while that of the control group was 556.0±15.68 micrometers](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0025208). The POAG group showed significantly higher IOP and significantly lower CCT in contrast to the control group (p<0.001 and 0.002, respectively). **Table 1**

The mean±SD MD of VF of POAG patients was -12.13 ± 2.42, while that of the control group was -0.72 ± 0.06. POAG group had more negative MD of VF in contrast to the control group (p<0.001). Regarding the severity of POAG patients, 52% of POAG patients had mild severity, 8% had moderate severity, 8% had advanced severity, and 32% had severe severity. **Table 1**

POAG group showed significantly lower vitamin D in contrast to the control group (p<0.001). The mean±SD vitamin D level in the POAG group was 27.16 ± 11.53, while that of the control group was 49.62 ± 11.42. **Table 1** and **Figure 1**

**Validity of vitamin D in the discrimination between POAG and controls:**

ROC analysis was done for discrimination between POAG and the control group. The area under the ROC curve (AUC) was 0.926 with a 95% CI of 0.844–1.000, which means that vitamin D is an excellent discriminator between POAG and the control group. The p-value was <0.001, which means that the result is significant. The cutoff value was ≤42.21 with a sensitivity of 96.0%, specificity of 88.0%, positive predictive value (PPV) of 88.89%, negative predictive value (NPV) of 95.65%, and accuracy of 92.0%. Vitamin D has high validity for the prediction of PAOG occurrence. **Figure 2**

**Validity of vitamin D in predicting severe and advanced disease:**

ROC analysis was done for the prediction of severe and advanced disease. [The AUC was 0.947 with a 95% confidence interval of 0.858–1.000 and a p-value <0.001](https://www.hindawi.com/journals/joph/2020/8071280/). [The cut-off value was ≤16.32 with a sensitivity of 80.0%, specificity of 100.0%](https://www.bing.com/search?form=MA13FV&OCID=MY02AA&pl=launch&q=Bing+AI&showconv=1), PPV of 100%, NPV of 88.24%, and accuracy of 92%. This indicates that vitamin D was valid for the prediction of advanced and severe POAG. **Figure 3**

**Prediction of POAG:**

Logistic regression analysis was done to predict POAG, using age, gender, and vitamin D level as confounders. The table shows that low vitamin D level was a significant predictor of POAG (OR<1, p<0.05). **Table 2**

**Prediction of POAG:**

Ordinal regression analysis was done to predict the severity of POAG, using age, gender, VA, CCT, IOP, and vitamin D level as confounders.

In univariable analysis, lower VA and vitamin D were linked to increased grades of severity (OR<1, p<0.05 for each).While in multivariable analysis, only vitamin D level was considered a predictor for higher grades of severity (OR<1, p<0.05). **Table 3**

**Discussion**

Recent research on serum vitamin functions in maintaining eye health, particularly with regard to the optic nerve and the severity of POAG, has been a subject of interest. Glaucoma encompasses a range of conditions that can affect the visual field and optic nerve, and it is not solely related to elevated intraocular pressure. Both primary and secondary causes can aid in the advancement of either open-angle or closed-angle glaucoma. POAG, the most prevalent type, often remains asymptomatic in its early stages, leading to late diagnosis and irreversible visual field loss (4, 8).

Racial heritage, a positive family background, advanced age, and elevated (IOP) are acknowledged POAG risk factors (11). Furthermore, because of vitamin D's impact on a number of physiological processes, including neurodefense inside the central nervous system —which includes the optic nerve—it has drawn interest in medical study. In addition, vitamin D regulates metabolic disorders such as dyslipidemia, diabetes, and hypertension, which are linked to a higher IOP and a lower ocular blood flow (12).

Despite the scarcity and inconclusiveness of research on the relationship between vitamin D and glaucoma, the immunosuppressive characteristics of vitamin D indicate its possible importance in safeguarding the optic nerve. Furthermore, it is possible that the influence of vitamin D on blood flow, specifically via the renin-angiotensin system, could play a role in its potential to prevent glaucoma.

This study's objective was to look into the link between the severity of POAG and serum vitamin D levels. The study included 25 patients with POAG from Banha University Hospitals alongside 25 healthy participants. The patients with POAG were divided into mild, moderate, advanced, and severe groups according to MD changes in the visual field: 52% had mild, 8% had moderate, 8% had advanced, and 32% had severe cases.

Serum vitamin D levels were measured in the POAG patient group and the control group. The findings indicated a significant variation in vitamin D levels between patients in the early and later phases of the disease. Mean 25-OH Vitamin D levels were 34.81 ± 6.66 in mild cases, 33.22 ± 7.08 in moderate cases, 29.33 ± 4.40 in advanced cases, and 12.66 ± 2.99 in severe cases, showing a clear decrease with increasing severity. Severe cases had notably lower vitamin D levels as opposed to other POAG grades.

According to the study, the mean±SD vitamin D level in the POAG group was 27.16 ± 11.53, while the control group's mean±SD vitamin D level was 49.62 ± 11.42. The POAG group had vitamin D levels less than those of the control group.

Previous studies agreed with our results and showed that the age-matched controls had a considerably greater amount of 25-OHD than the POAG. According to two investigations, age-matched controls had higher serum levels of 1a, 25-dihydroxyvitamin than POAG patients did (13, 14).

Our results are in line with a large cross-sectional study of South Koreans, which comprised 290 instances of OAG, 410 probable cases of glaucoma, and 5,394 controls. The study's discovery that OAG risk was significantly higher in those with lower vitamin D levels provided evidence for the link between vitamin D levels and glaucoma (3).

A French study also discovered lower vitamin D levels in glaucoma patients. Patients with mild POAG and those with severe POAG did not differ in their serum vitamin D levels, according to that study. The differences in glaucoma severity may be explained by the differences between the two glaucoma groups that were analyzed and by the results of the current investigation regarding the relationship between vitamin D and glaucoma severity (15).

Other research conducted in Croatia has shown that glaucoma patients' serum vitamin D levels are lower than those of normal controls (10). A study also found the same results (16).

Additionally, participants in this retrospective, cross-sectional study completed a health survey at the Kangbuk Samsung Hospital's Health Screening Center. The study's findings indicated that, in comparison to a higher 25(OH)D level, a lower 25(OH)D level was substantially connected to a higher incidence of glaucoma in women (9).

A study found that OAG risk and 25(OH)D levels possess a strong positive relationship, with a negative correlation seen at lower levels. The results of the study indicate that vitamin D insufficiency has to be taken into account as a possible risk factor for the emergence of OAG (10), which also agrees with our results.

A different study discovered that the normal control group's serum-free vitamin D levels ranged from 1.08 to 33.64 pg/ml, with an average ± standard deviation of 8.02 ± 6.19 pg/ml. Within the range of 0.77–33.22 pg/ml, patients in the early POAG group had free vitamin D levels with a mean ± SD of 7.56 ± 5.74 pg/ml. The serum-free vitamin D levels of the patients with advanced glaucoma, on the other hand, were lower, ranging from 0.59 to 31.47 pg/ml, with a mean ± SD of 6.35 ± 4.76 pg/ml. Blood vitamin D levels were thus considerably less in advanced glaucoma patients than in the normal control and early glaucoma groups (4).

On the other hand, glaucoma patients' serum vitamin D levels did not alter statistically significantly from those of control people in another Turkish case-control study (17).

The study's findings showed that the blood levels of 25-hydroxyvitamin D in the POAG group were 72.58 ± 31.79 nmol/L, whereas the PACG group had levels of 69.20 ± 24.24 nmol/L, and the control group had values of 67.14 ± 29.02 nmol/L. These results were not in line with our observations. Vitamin D levels were typically poor in the majority of participants across all categories. While there were no statistically significant variations in the serum levels of 25-OHD between the three investigated groups, the POAG group's individuals had a borderline acceptable amount of vitamin D, while the controls had the lowest level. This was succeeded by the PACG group (18).

Nevertheless, no discernible variations in serum vitamin D levels were observed between POAG and controls in a study evaluating the relationship between vitamin D and various forms of glaucoma, including POAG, in the Chinese population (19).

Comparably, there is no meaningful relationship between the prevalence of glaucoma and diet, supplements, or serum vitamin D levels, according to a recent large-sample study carried out in the US (20).

The discrepancy between the results of the current study and those from earlier research could be attributed to participant ages and ethnicity. This suggests that lowering the blood 25-OHD level was not caused by age as a variable factor. However, it is well known that older adults are more likely to have vitamin D shortage because aging lowers skin concentrations of 7-DH (7 Dehydrocholesterol), which lowers the skin's capacity to produce vitamin D (21).

The relatively small research population and the single-center nature are the main limitations of our study.

**Conclusion**

In conclusion, there has been evidence over the past few decades linking vitamin D to eye conditions like glaucoma. It is thought to work by regulating the immune system and preventing inflammation and angiogenesis to provide protection against a number of eye illnesses or disorders. Thus, vitamin D insufficiency might be a key factor in the etiology of glaucoma, influencing its onset, course, and severity. The current investigation discovered that there was a strong relationship between the severity of the sickness and serum vitamin D levels and that a vitamin D deficit was a good indicator of the incidence of POAG.

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**Author contribution:**

Each author made an equal contribution to the research.

**Conflicts of interest:**

Absence of conflicts of interest.

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**Table 1: General, clinical, and laboratory characteristics of the studied groups**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **POAG**  **N = 25** | | **Control**  **N = 25** | | **Test** | **P** |
| **Age (years)** |  | |  | |  |  |
| Mean ± SD. | 49.68 ± 11.58 | | 42.16 ± 15.76 | | t= 1.923 | 0.061 |
| **Sex** | *No.* | *%* | *No.* | *%* |  |  |
| Male | 14 | 56.0 | 10 | 40.0 | X2= 1.282 | 0.258 |
| Female | 11 | 44.0 | 15 | 60.0 |
| **Visual acuity** |  |  |  |  |  |  |
| Median (range) | 0.5 (0.2 – 0.8) | | 0.8 (0.4 – 1) | | U=131 | <0.001\* |
| **IOP (mmHg)** |  |  |  |  |  |  |
| Mean ± SD. | 18.80±3.46 | | 14.96±3.06 | | T=4.153 | <0.001\* |
| **CCT** |  |  |  |  |  |  |
| Mean ± SD. | 533.1 ± 31.45 | | 556.0±15.68 | | T=3.261 | 0.002\* |
| **MD of VF** |  |  |  |  |  |  |
| Median (range) | -5.20 (-35.52 – 1.2) | | -0.60 (-1.4 - -0.37) | | U= 565.0\* | <0.001\* |
| **Severity** | *No.* | *%* | *No.* | *%* |  |  |
| Mild | 13 | 52.0 | - | - | - | - |
| Moderate | 2 | 8.0 | - | - |  |  |
| Advanced | 2 | 8.0 | - | - |  |  |
| Severe | 8 | 32.0 | - | - |  |  |
| **Vitamin D level** |  |  |  |  |  |  |
| Mean ± SD. | 27.16 ± 11.53 | | 49.62 ± 11.42 | | t= 6.920\* | <0.001\* |

U: Mann–Whitney; t: Student t test; X2:Chi–Square, P: Comparing POAG and control group; \* Significant when p<0.05; SD: Standard deviation.

**Table 2: Logistic Regression analysis for the predictors of POAG.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **P** | **OR** | **95% CI.** |
| **Age** | 0.065 | 1.041 | 0.998–1.086 |
| **Gender** | 0.260 | 1.909 | 0.620–5.876 |
| **Vitamin D level** | <0.001\* | 0.920 | 0.886-0.955 |

OR: Odd Ratio; CI, confidence interval. \*: Significant when p value <0.05.

**Table 3: Ordinal Regression analysis for the predictors of severity of POAG.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Univariate** | | | **Multivariate** | | |
| **P** | **OR** | **95% CI** | **P** | **OR** | **95% C.I.** |
| **Age** | 0.973 | 0.999 | 0.958-1.042 |  |  |  |
| **Gender** | 0.479 | 0.713 | 0.280-1.816 |  |  |  |
| **Visual acuity** | 0.007\* | 0.010 | 0.002-0.282 | 0.744 | 0.499 | 0.008-3.120 |
| **IOP** | 0.114 | 1.122 | 0.973-1.294 |  |  |  |
| **CCT** | 0.335 | 1.007 | 0.992-1.022 |  |  |  |
| **Vitamin D level** | <0.001\* | 0.878 | 0.825-0.934 | 0.001\* | 0.858 | 0.786-0.983 |

OR: Odd Ratio; CI, confidence interval. \*: Significant when p value <0.05.



**Figure 1: Column chart for comparison between POAG and control group regarding vitamin D level.**

A graph of vitamin d level

Description automatically generated

**Figure 2: ROC Curve for vitamin D level for discrimination between POAG and control group.**

A diagram of vitamin d level

Description automatically generated

**Figure 3: ROC Curve for vitamin D level for prediction between (mild& moderate) POAG versus (advanced& severe) POAG.**