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Plasma 25-Hydroxyvitamin-D (25-OHD) levels and late-stage age-related macular degeneration (AMD): Assessing the correlation

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ABSTRACT

Background: Age-related Macular Degeneration (AMD) is a degenerative disease caused by multiple factors, including inflammation. This condition may lead to irreversible macular damage. Vitamin D has anti-inflammatory and immune-modulating qualities, therefore, is expected to protect against the development of AMD.
Objective: This study aimed to evaluate the correlation between plasma 25-hydroxyvitamin D (plasma 25-OHD) levels and neovascular Age-Related Macular Degeneration (nAMD).
Methods: This was a case-control study involving AMD and control subjects. All subjects underwent a standardized eye examination to check for eligibility by a retinal specialist. Blood samples were drawn for analysis using a DRG Elisa Kit to determine the plasma 25-OHD (Total). Plasma 25-OHD levels were categorized into deficiency (<12 ng/mL), insufficiency (12 - <20 ng/mL), adequate (20 – 50 ng/mL), and high (>50 ng/mL).
Results: Total subjects were 39, which consisted of 20 AMD subjects and 19 controls. There were 17 male subjects (43.6%) and 22 female subjects (56.4%). The mean age for the case and control group was 69.35±7.04 and 68.26±6.83, respectively (p=0.612). Those in their 70s dominated the age distribution with a percentage of 56.41% of all participants. The mean plasma 25-OHD in the case group was 32.30±17.10 ng/mL, while the control group was 50.63±11.95 ng/mL (p=0.066). There was no significant association between AMD and plasma 25-OHD levels in all groups (p>0.05).
Conclusion: Plasma 25-OHD levels did not correlate positively with nAMD in our study population.

Latar belakang: *Age-related Macular Degeneration (AMD) adalah kondisi degeneratif yang menyebabkan kerusakan makula secara ireversibel. AMD disebabkan oleh banyak faktor, termasuk inflamasi. Vitamin D memiliki kemampuan sebagai anti-inflamasi dan pengaktifan sistem imun, sehingga diharapkan dapat membantu mencegah perjalanan penyakit AMD.*

Tujuan: *Penelitian ini bertujuan untuk mengevaluasi hubungan antara kadar plasma 25-hydroxyvitamin-D (plasma 25-OHD) dengan neovaskular Age-related Macular Degeneration (nAMD).*

Metode: *Penelitian ini merupakan studi kasus kontrol yang melibatkan subjek kontrol dan AMD. Seluruh subjek melalui pemeriksaan mata yang terstandar untuk memastikan eligibilitas yang dilakukan oleh spesialis retina. Sampel darah diambil untuk analisis. DRG Elisa Kit digunakan untuk menentukan kadar total 25-OHD pada plasma sampel. Kadar 25-OHD pada plasma dikelompokkan menjadi defisiensi (<12 ng/mL), insufisiensi (12 - <20 ng/mL), cukup (20 – 50 ng/mL), dan tinggi (>50 ng/mL).*

Hasil: *Jumlah subjek yaitu sebanyak 39, terdiri dari 20 subjek AMD dan 19 kontrol. Terdapat 17 subjek laki-laki (43.6%) dan 22 subjek perempuan (56.4%). Usia rata-rata untuk kelompok kasus dan kontrol yaitu 69.35±7.04 dan 68.26±6.83 secara berurutan (p=0.612). Subjek dengan usia sekitar 70 tahun mendominasi distribusi usia dengan persentase 56.41% dari seluruh partisipan. Rata-rata kadar 25-OHD pada kelompok kasus adalah. 32.30±17.10 ng/mL, sementara pada kelompok kontrol sebesar 50.63±11.95 ng/mL (p=0.066). Tidak ada hubungan yang signifikan antara AMD dan kadar 25-OHD plasma pada semua kelompok (p>0.05).*

Kesimpulan: *Kadar 25-OHD pada plasma tidak berkorelasi secara positif dengan nAMD pada populasi studi.*

INTRODUCTION

Age-related Macular Degeneration (AMD) is a degenerative condition that causes irreversible macular damage. This condition causes central vision loss even when the peripheral vision is still performing well, thus causing difficulties in daily activities. There are approximately 50 million people who suffer from AMD worldwide, and 8 million of them are in the United States.[1] In the Europe population, the prevalence of early AMD was 25.3%, and late AMD was 2.4%, with the majority from the age of 60 and older.[2] The Australian National Eye Health Survey reported that the prevalence of both early and late AMD at the age of 40 until 98 years in indigenous Australian was 13.8% and 0.17%, respectively.[3] Unfortunately, there are currently no reliable data about the incidence or the prevalence of AMD in Indonesia. However, the incidence of AMD is predicted to increase following the growing elderly population.

AMD is caused by multiple factors, including inflammation, which contributes to degenerative development. Inflammation could be a potent risk factor for AMD.[4] Many studies regarding the role of inflammation in the pathogenesis of AMD. Therefore, more studies are being conducted to find a compensation pathway to relieve inflammation, such as vitamin D.

Vitamin D has anti-inflammatory and immune-modulating qualities, and many speculate that the vitamin can protect against the development of AMD. Research suggests that vitamin D impacts immune modulation and could prevent diseases with inflammatory etiologies.[5,6] Furthermore, in vivo study indicated that the major circulating metabolite of vitamin D (25-hydroxyvitaminD[25(OH)D]) was converted by not only the proteins for the vitamin D receptor (VDR) but also the enzyme 1-α-hydroxylase to its active hormone calcitriol (1,25-dihydroxy vitamin D), which expressed in the retina.[5,6] Hence, this study investigated the association between plasma 25-OHD levels and AMD.

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METHODS

Subject recruitment was centered on dr. Sardjito Hospital and dr. Suhardi Hardjolukito Hospital, Yogyakarta from August 2016 to November 2018. All subjects were age-matched, preferably aged 45 years old or older and without prior AMD treatment. All underwent standardized eye examination consisted of visual acuity (VA), slit-lamp examination, fundus photographs, and spectral-domain ocular coherence tomography (OCT). nAMD diagnosis was confirmed by a retinal specialist following the standard examination and AMD classification criteria.[7]Additional medical information such as hypertension, cigarette smoking, body weight, and body height were taken from the medical records or by direct anamnesis.

This study adhered to the declaration of Helsinki and was approved by the Ethical Committee of FK-KMK UGM with the number KE/FK/864/EC [5 August 2016] before the beginning of the research. All subjects received adequate information before deciding to participate in the study and were aware of the study course. Written informed consent was obtained from all subjects before participation.

ELISA analysis

Blood samples were drawn (approx. 3 mL) from each subject for ELISA analysis. Samples were stored at 2°C - 8°C before assaying. All specimens were immediately assayed after collection. A plasma 25-OH Vitamin D (Total) ELISA kit from DRG Instruments GmbH (DRG Instruments GmbH, Germany) was used in this study. Assay procedures were according to the attached protocols from the manufacturer.

Data Analysis

History of cigarette smoking and hypertension were categorized into yes or no. Body mass index (BMI) was calculated using the standard BMI formula

$$\left(\frac{bodyweight\ in\ kg}{(bodyheight\ in\ m)^2}\right) \quad \textbf{(1)}$$

and further classified according to the Asian standard BMI. Plasma 25-OHD levels were categorized into four groups according to the Institute of Medicine, Food and Nutrition Board year 2010, which are: deficiency (<12 ng/mL), insufficiency (12 - <20 ng/mL), adequate (20 -50 ng/mL), and high (>50 ng/mL).[8]

Statistical analysis using IBM SPSS Software Version 23 using Student's t, the Mann-Whitney non-parametric test, χ^2, logistic regression, and correlation tests with 95% Confident Interval (CI).

RESULTS

The subject was 39 in total, which consisted of 20 AMD subjects and 19 controls. There were 17 male subjects (43.6%) and 22 female subjects (56.4%). In the AMD group, 18 subjects (90%) had wet AMD, while the other two subject (10%) had both wet and dry AMD. Mean logMAR VA in the control group for the right and left eye was better compared to the AMD group. However, the difference was not significant (p>0.05). (Table 1)

The mean age for the case and control group was 69.35±7.04 (range: 53 – 79 years old) and 68.26±6.83 (range: 53 – 77 years old), respectively (p=0.612). Those in the 70s

dominated the age distribution with a percentage of 56.41% of all participants. The mean plasma 25-OHD in the case group was 32.30±17.10 (range: 8.57 – 66.74 ng/mL), while the control group was 50.63±11.95 (range: 0.32 – 63.96 ng/mL). However, the difference was not significant (p=0.066). Hypertension was significantly associated with AMD (p=0.029) but not cigarette smoking (p=0.798), sex (p=0.140), and BMI (p>0.05). (Table 1)

Table 1. Baseline characteristics of the subjects

Variables	Subjects (n=39)		p	OR
	Case (n=20)	Control (n=19)		
Mean age ± SD, year*	69.35 ± 7.04	68.26 ± 6.83	0.612	-
Mean plasma 25-OHD ± SD, ng/mL*	32.20 ± 17.10	22.91 ± 13.71	0.066	-
Mean bodyweight ± SD, kg*	60.50 ± 14.85	50.63 ± 11.95	0.022	-
Mean body height ± SD, cm*	160.20 ± 8.69	151.11 ± 9.24	0.006	-
Mean BMI ± SD, kg/m2*	23.37 ± 4.21	22.19 ± 5.14	0.187	-
Mean RE VA logMAR ± SD	0.96 ± 0.55	0.68 ± 0.49	0.086	-
Mean LE VA logMAR ± SD	1.15 ± 0.89	0.78 ± 0.58	0.284	-
Age groups, year (%)†				
50 – 59	2 (10.0%)	3 (15.8%)	Ref	Ref
60 – 69	6 (30.0%)	6 (31.6%)	0.746	0.68 (0.06 – 6.26)
70 – 79	12 (60.0%)	10 (52.6%)	0.602	0.57 (0.05 – 4.53)
Sex (%)†				
Male	11 (55.0%)	6 (31.6%)	0.140	0.38 (0.10 – 1.39)
Female	9 (45.0%)	13 (68.4%)		
Cigarette smoking (%)†				
Yes	6 (30.0%)	5 (26.3%)	0.798	1.20 (0.29 – 4.86)
No	14 (70.0%)	14 (73.7%)		
Hypertension (%)†				
Yes	11 (55.0%)	4 (21.1%)	0.029	4.58 (1.11 – 18.80)
No	9 (45.0%)	15 (78.9%)		
BMI groups (%)†				
Underweight (<18.5 kg/m^2)	7 (35.0%)	9 (47.4%)	0.543	0.53 (0.06 – 3.62)
Normal (18.5 – 22.9 kg/m^2)	2 (10.0%)	5 (26.3%)	Ref	Ref
Overweight (23 – 24.9 kg/m^2)	5 (25.0%)	2 (10.5%)	0.157	0.18 (0.01 – 1.81)
Obese (≥25 kg/m2)	6 (30.0%)	3 (15.8%)	0.177	0.22 (0.02 – 1.88)
Level of plasma 25-OHD (%)†				
Deficiency (<12 ng/mL)	1 (5.0%)	4 (21.1%)	0.282	3.8 (0.39 – 108.1)
Insufficiency (12 - <20 ng/mL)	5 (25.0%)	4 (21.1%)	0.799	0.81 (0.15 – 4.13)
Adequate (20 – 50 ng/mL)	10 (50.0%)	10 (52.6%)	Ref	Ref
High (>50 ng/mL)	4 (20.0%)	1 (5.3%)	0.283	0.26 (0.009 – 2.53)

*Mann-Whitney non-parametric test
†Chi-square test
N: number of subjects; SD: standard deviation; ng: nanogram; mL: millilitre; OD: odds ratio; kg: kilogram; cm: centimetre; Ref: reference; RE: right eye; LE: left eye; VA: visual acuity

There was a total of 20 subjects (51.28%) who had an adequate level of plasma 25-OHD, five subjects (12.82%) in the deficiency group, nine subjects (23.07%) in the insufficiency group, and five subjects (12.82%) in the high group. In both AMD and control groups, majority of the subjects had an adequate levels of plasma 25-OHD with an average percentage of 50% of all subject. However, the level of plasma 25-OHD did not correlate positively with AMD in all the

Table 2. Bivariate analysis of plasma 25-OHD using regression test

Variable	Case (n=20)	Control (n=19)	Unadjusted p	Unadjusted OR	Adjusted p*	Adjusted OR*
Level of plasma 25-OHD (%)						
Deficiency (<12 ng/mL)	1 (5.0%)	4 (21.1%)	0.250	0.25 (0.02 – 2.65)	0.380	0.34 (0.03 – 3.85)
Insufficiency (12 - <20 ng/mL)	5 (25.0%)	4 (21.1%)	0.782	1.25 (0.26 – 6.07)	0.582	1.61 (0.29 – 8.69)
Adequate (20 – 50 ng/mL)	10 (50.0%)	10 (52.6%)	Ref	Ref	Ref	Ref
High (>50 ng/mL)	4 (20.0%)	1 (5.3%)	0.250	4.00 (0.38 – 42.36)	0.306	3.62 (0.31 – 42.46)

*adjusted by age and sex

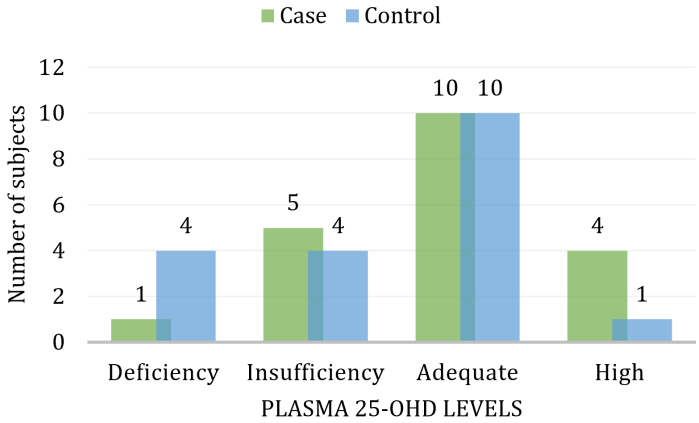

Figure 1. Distribution of plasma 25-OHD levels in both study subjects

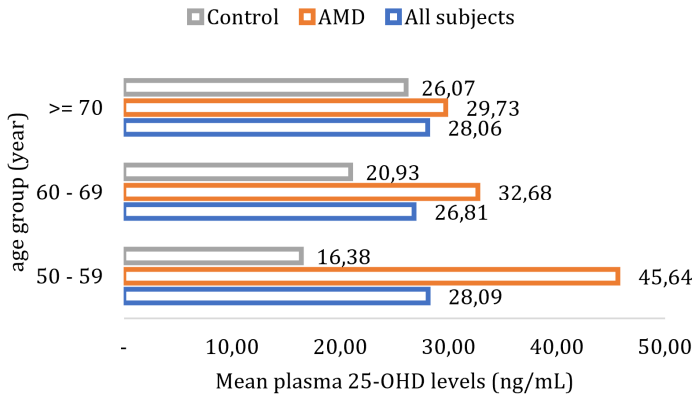

Figure 2. Comparison of mean plasma 25-OHD levels between age groups

classification groups (p>0.05). Further analysis using a regression test showed a consistent insignificant result between plasma 25-OHD levels and AMD, even after adjusting the gender and sex (p>0.05). (Table 2 and Figure 1)

Mean plasma 25-OHD levels between each age group in all subjects showed a similar value about 26 – 28 ng/ml. However, a downward trend was visible in the AMD group where the older the age group, the smaller the mean 25-OHD was. In the age group 50 – 59 years, the mean 25-OHD level was about 45.64 ng/ml, while in the group ≥70 years was 29.73 ng/ml. Conversely, an upward trend was found in the control group where the older the age group, the higher the mean 25-OHD plasma was. In groups ≥70 years, the mean was 26.07 ng/ml, while the mean value in the 50 – 59 group was 16.38 ng/ml. (Figure 2)

DISCUSSION

The study result indicated that the mean plasma 25-OHD level was higher in the AMD group compared to the control group. However, the result was not statistically significant with a downward trend of mean plasma 25-OHD levels present as the older the AMD subjects became. Yet, still no association found between plasma 25-OHD levels and late-stage AMD. A small sample size and a simple study design might be the reason for this result as each study group might not be well represented. The case-control study may have potential methodological limitations as it is hard to find the exact causality between plasma 25-OHD and AMD. However, several other factors may have affected the result of the study, such as vitamin D supplements, food and calcium intake, the amount of sun exposure, and patient's genetic mutation.

This study evaluated those with late-stage AMD, either wet or dry or both dry and wet AMD versus controls. Therefore, we could not predict the risk for the early stage of AMD. Plasma 25-OHD levels tended to be of adequate level in this study. Again, the small sample size and confounding factors might be the reason.

Similar studies had been done previously to see whether Vitamin D correlates positively with AMD through the evaluation of plasma 25-OHD levels. The result varied across the high concentration, low concentration, and no association with the disease onset. A study in South Korea reported that Vitamin D deficiency increased the risk of early AMD (OR 3.59 [0.95 – 13.58]; 95% CI; p=0.060) and was significantly associated with a greater risk of late AMD (OR=3.61; 95% CI 1.04 – 12.51; p=0.043).[9] A similar result was reported in US and Turkish population, low circulated vitamin D (<70nM) was correlated with early AMD.[10, 11]

Another study found that a low serum level of 25-OHD was associated with early AMD, with the highest quintile serum 25D3 levels decreasing the prevalence of early AMD, but not for late-stage AMD.[12,13] On the contrary, low levels of Vitamin D (<50nmol/L) are associated with late-stage AMD.[14] A study in Spain shows no association between vitamin D with any stages of AMD.[15]

Vitamin D is a known protective factor against inflammation, oxidative stress, fibrosis, and angiogenesis which usually occurs in AMD. Therefore, many hypothesized to use of Vitamin D as a therapy against AMD. Data from Tohari et al. suggested that Vitamin D suppresses RPE cell inflammation and oxidative damage, hence supporting the evidence of vitamin D's roles in AMD.[16] Inflammation and angiogenesis in AMD's retina could be protected by Vitamin D supplementary, yet the mechanism remains unclear.[17]

CONCLUSION

In conclusion, though the result showed that Vitamin D did not correlate positively with nAMD in this study, Vitamin D is still a potential substance to study especially for its use in AMD therapy. Further study with a larger sample size to represent the wider population is necessary to better understand the correlation between Vitamin D and late-stage AMD in Indonesia.

CONFLICT OF INTEREST

The authors hereby declared that there was no conflict of interest from any third-party organizations throughout the course of the study. The study received funding from the Community Research Fund (Hibah Dana Masyarakat) by FK-KMK UGM year 2017. The authors independently carried out this study without any interference from the funding body.

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