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Effect of Vitamin D Supplementation on Irisin, Telomerase, Klotho, and Tumor Necrosis Factor-alpha (TNF-α) in Elderly: A Quasi-Experimental Study

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Abstract

Background: Vitamin D plays a crucial role in aging by regulating mitochondrial function, inflammation, oxidative stress, and telomere stability. Vitamin D deficiency is common among the elderly and is linked to accelerated aging. Biomarkers such as irisin, telomerase, klotho, and tumor necrosis factor-alpha (TNF-α) are associated with aging processes. This study aimed to evaluate the effect of vitamin D supplementation on these biomarkers in elderly individuals.

Methods: This quasi-experimental pretest-posttest study was conducted in Kadugadung Village, Banten, Indonesia from March to September 2024. A total of 47 healthy elderly individuals (≥60 years) were recruited using purposive sampling. The treatment group (n=25) received 800 IU/day of vitamin D for 20 days, whereas the control group (n=22) received none. Blood samples were collected before and after the intervention to measure serum irisin, telomerase activity, klotho, and TNF-α. Baseline variables included body mass index (BMI), blood pressure, hemoglobin, hematocrit, blood glucose, cholesterol, and uric acid. Data were analyzed using paired and independent statistical tests.

Results: Vitamin D supplementation significantly increased serum irisin levels ($p=0.016$), meanwhile no significant changes were observed in telomerase activity ($p=0.128$), klotho ($p=0.819$), or TNF-α ($p=0.098$). In the treatment group, blood glucose was correlated positively with TNF-α ($r=0.423$, $p<0.05$), whereas cholesterol was correlated negatively with TNF-α ($r=-0.51$, $p<0.01$). Furthermore, telomerase activity was correlated positively with irisin ($r=0.348$, $p<0.05$).

Conclusions: Vitamin D supplementation at 800 IU/day significantly enhances serum irisin, but does not affect telomerase, klotho, or TNF-α. These findings suggest a potential role of vitamin D in modulating aging-related biomarkers.

Keywords: Irisin, klotho, telomerase, TNF-α, vitamin D

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Introduction

Aging is a biological process involving the progressive accumulation of molecular and cellular damage, contributing to deterioration in physical and cognitive function, increased disease risk, and mortality. It is characterized by mitochondrial dysfunction, epigenetic modifications, and telomere shortening.[1] Nutritional patterns, particularly vitamin D

intake, play an important role in modulating these process.[2] Vitamin D deficiency, defined as serum levels below 50 nmol/L or 20 ng/mL, is a global health issue that primarily affects the elderly population.[3,4] In Indonesia, 50% of women aged 45–55 years are vitamin D deficient.[5] Contributing factors include insufficient dietary intake, limited sun exposure, reduced vitamin D production in the body, and increased catabolism in the liver.[6]

Vitamin D is involved in multiple aging-related pathways, including autophagy, mitochondrial dysfunction, inflammation, oxidative stress, epigenetic modifications, DNA damage repair, and calcium (Ca^{2+}) and reactive oxygen species (ROS) regulation.[7] Several biomarkers provide insights into these mechanism. Irisin, a myokine with anti-inflammatory and bone-protective properties, declines with age and is enhanced by vitamin D.[8,9] Telomere shortening promotes cellular senescence and apoptosis, but can be mitigated by telomerase activity, which vitamin D may regulate through human telomerase reverse transcriptase (hTERT) expression and anti-inflammatory mechanisms.[10,11] The anti-aging protein Klotho, primarily expressed in the kidneys, also decreases with age and is influenced by vitamin D levels.[12,13] In addition, chronic low-grade inflammation, often indicated by elevated tumor necrosis factor-alpha (TNF-α), contributes to aging. Vitamin D may counteract TNF-α-induced inflammation by regulating mitochondrial dynamics and the AKT/NF-κB pathway.[14.15]

Although previous studies have explored individual associations between vitamin D and specific biomarkers, few have evaluated the simultaneous effects of vitamin D supplementation on multiple cellular indicators of aging, leaving little understanding of the interactions between these biomarkers. Therefore, this study assessed the effects of vitamin D supplementation on irisin, telomerase, klotho, and TNF-α in elderly individuals, aiming to investigate the impact of vitamin D supplementation on these biomarkers and examine their associations in the context of healthy aging.

Methods

This study employed a quasi-experimental one-group pretest-post-test design. Tests were conducted on participants before (pretest) and after (post-test) treatment. The study was carried out in Kadugadung Village, Pandeglang District, Banten Province, Indonesia, from March to September 2024. Elderly individuals (≥60 years) were recruited using purposive sampling. Inclusion criteria were good health without comorbidities (hypertension, diabetes mellitus), no prior vitamin D supplementation, and willingness to participate. Exclusion criteria were illness during the supplementation period or discontinuation of vitamin D supplementation. The study protocol was approved by the Ethics Committee of YARSI University (No. 287/KEP-UY/EA.20/IX/2024).

The treatment group received vitamin D supplementation (Nature Plus) at a dosage of 800 IU for 20 days, whereas the control group did not. Venous blood samples were collected from fasting participants at baseline and after the intervention. Baseline measurements

Table 1 Baseline Characteristics of Elderly Individuals Who Received Vitamin D Supplementation as Treatment Group Compared to Control Group

Characteristic	Group		p-value
	Treatment (n=25)	Control (n=22)	
	Mean±SD	Mean±SD	
Weight (kg)	52.77±8.82	56.09±9.34	0.217[a]
Height (cm)	150.86±5.12	154.27±7.43	0.100[b]
Body mass index (kg/m^2)	23.25±3.99	23.68±4.31	0.728[a]
Systolic blood pressure (mmHg)	139.92±28.05	133.59±17.86	0.369[a]
Diastolic blood pressure (mmHg)	74.80±8.01	75.23±9.36	0.653[b]
Blood glucose (mg/dL)	135.64 ±44.65	149.68±48.49	0.114[b]
Cholesterol level (mg/dL)	192.56±43.64	184.00±29.05	0.439[a]
Uric acid level (mg/dL)	5.42±1.82	5.43±1.51	0.974[a]
Hemoglobin level (g/dL)	12.41±1.42	12.06±1.77	0.463[a]
Hematocrit (%)	37.24±4.23	36.18±5.35	0.453[a]

Note: a=independent t-test, b=Mann-Whitney test

Table 2 Comparison of Serum Irisin, Telomerase Activity, Klotho, and TNF-α Levels Among Elderly Individuals who Received Vitamin D Supplementation as Treatment Group and Control Group

Variable	Group	Pre-test Mean ± SD	Post-test Mean ± SD	p-value (within group)	p-value (between groups)
Irisin (ng/mL)	Treatment	27.02 ± 13.32	43.24 ± 24.44	0.016[c]*	0.001[b]*
	Control	24.51 ± 16.99			0.141[b]
Telomerase (TPG)	Treatment	1.74 ± 1.15	1.75 ± 1.11	0.128[c]	0.015[b]*
	Control	1.06 ± 0.80			0.023[b]*
Klotho (pg/mL)	Treatment	1334.3 ± 1711.8	1010.8 ± 396.4	0.819[c]	0.001[b]*
	Control	1967.3 ± 1114.6			0.001[a]*
TNF-α	Treatment	220.1 ± 169.6	165.0 ± 116.1	0.098[c]	0.082[b]
	Control	226.8 ± 188.8			0.717[b]

Note: Data were presented as mean ± standard deviation. Within-group comparisons were performed using Wilcoxon signed-rank test (c). Between-group comparisons were performed using independent t-test (a) or Mann–Whitney test (b). *p<0.05

included body mass index (BMI), blood pressure, hemoglobin, hematocrit, blood glucose, cholesterol, and uric acid. BMI was measured using a microtoise for height and a calibrated digital scale for weight. Hemoglobin and hematocrit were measured with a Family Dr. hemoglobin meter.

Serum irisin, telomerase, klotho, and TNF-alpha were measured before and after vitamin D supplementation. Venous blood samples were centrifuged, and serum was transported to the Integrated Laboratory of YARSI University, Indonesia. Biomarker levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (Bioenzy).

Quantitative variables were expressed as mean±standard deviation. Comparison between pretest and posttest data were performed using the paired t-test for normally distributed data and Wilcoxon signed-rank test for non-normal data. The Shapiro–Wilk test was used to assess normality. A 95% confidence level was applied, and analyses were conducted using IBM SPSS version 26.0.

Results

A total of 47 elderly individuals (≥60 years) were included; the treatment group (n=25) had received vitamin D supplementation at a dosage of 800 IU for 20 days, whereas the control group (n=22) did not. The baseline clinical characteristics of the participants were shown in Table 1. Overall, the treatment

Table 3 Correlation between Baseline Characteristics and Biomarker Levels in the Treatment Group

Characteristic	Biomarker							
	Telomerase		Irisin		Klotho		TNF-α	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
BMI	0.368	0.387	0.058	0.173	0.087	-0.104	0.232	0.219
Systolic blood pressure	0.044	0.008	0.391	-0.030	0.245	0.230	0.090	0.134
Diastolic blood pressure	0.074	0.066	0.157	0.038	-0.079	0.234	-0.267	-0.279
Blood sugar level	0.189	0.198	0.177	0.210	0.067	0.109	0.423	0.346
Cholesterol level	0.238	0.217	0.112	0.058	0.135	0.105	-0.510	0.059
Uric acid level	0.032	0.000	0.121	0.141	0.279	0.075	-0.150	-0.085
Hemoglobin level	-0.114	-0.100	0.012	-0.251	0.072	0.134	0.019	0.182
Hematocrit	-0.107	-0.094	0.013	-0.251	0.080	0.134	0.028	0.181

Note: Values represent Spearman's correlation coefficients

Table 4 Correlation between Baseline Characteristics and Biomarker Levels in the Control Group

Characteristic	Biomarker							
	Telomerase		Irisin		Klotho		TNF-α	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
BMI	-0.057	-0.057	-0.055	-0.055	-0.474	-0.474	0.069	0.069
Systolic blood pressure	0.049	0.049	0.327	0.327	-0.092	-0.092	-0.327	-0.327
Diastolic blood pressure	0.146	0.146	0.244	0.244	-0.062	-0.062	-0.251	-0.251
Blood sugar level	-0.168	-0.168	0.062	0.062	-0.313	-0.313	0.098	0.098
Cholesterol level	0.194	0.194	0.461	0.461	-0.073	-0.073	-0.197	-0.197
Uric acid level	0.156	0.156	-0.041	-0.041	0.026	0.026	0.277	0.277
Hemoglobin level	-0.179	-0.179	0.185	0.185	-0.280	-0.280	-0.259	-0.259
Hematocrit	-0.171	-0.171	0.191	0.191	-0.287	-0.287	-0.261	-0.261

Note: Values represent Spearman's correlation coefficients

Table 5 Correlation Between Parameters

Parameter	Telomerase Activity	Serum Irisin	Serum Klotho	Serum TNF-α
Telomerase activity	1.000	0.348*	-0.176	-0.094
Serum irisin	-	1.000	-0.290	-0.295
Serum klotho	-	-	1.000	0.055
Serum TNF-α	-	-	-	1.000

Note: *) significant level <0,05

group had higher mean values of body mass index (BMI), blood pressure, cholesterol, hemoglobin, and hematocrit compared with the control group, while the control group had higher mean values of blood glucose and uric acid. None of these differences reached statistical significance.

Normality testing of serum biomarkers using the Shapiro–Wilk test indicated non-normal distribution. Therefore, Wilcoxon signed-rank tests were applied. In the treatment group, vitamin D supplementation significantly increased serum irisin levels (p=0.016). No significant changes were observed in telomerase activity (p=0.128), serum klotho (p=0.819), or tumor necrosis factor-alpha (TNF-α) (p=0.098). Between groups, serum irisin levels were significantly higher in the treatment group post intervention (p=0.001) (Table 2).

Correlation analyses were performed to assess the relationships between baseline clinical variables and biomarker levels. In the treatment group, blood glucose correlated positively with TNF-α (r=0.423, p<0.05), while cholesterol correlated negatively with TNF-α (r=-0.510, p<0.01) (Table 3 and 4). These associations were not observed in the control group.

Analysis of relationships among the primary biomarkers showed a significant positive correlation between telomerase activity and serum irisin levels (r=0.348, p<0.05). This finding suggests potential interaction between telomerase function and irisin in aging-related pathways (Table 5).

Discussion

Irisin, secreted by skeletal muscles, regulates metabolism and reduce inflammation during aging. Physical activity increases irisin levels, helping to maintain mitochondrial function, energy balance, and protect muscles and cognition from age-related degeneration.[16] Vitamin D also influences muscle health through the vitamin D receptor (VDR), PGC-1α, and the p38/MAPK pathway, which are linked to FNDC5 expression, the precursor of irisin.[17,18] In this study, vitamin D supplementation significantly increased serum irisin levels. This finding aligns with previous trials reporting elevated serum irisin after high-dose vitamin D (50,000 IU per week for

eight weeks) in patients with type 2 diabetes.[9] Interestingly, the effect was observed with a much lower daily dose (800 IU/day or 5,600 IU/week) compared to previous study (e.g., 50,000 IU/week), suggesting that modest supplementation may still promote irisin synthesis in elderly individuals. Differences in baseline vitamin D status, treatment duration, and population characteristics may explain variability in responsiveness. Further research is needed to clarify the dose–response relationship.[19]

Telomeres maintains telomere length and genomic stability, and its deficiency accelerates cellular aging.[20,21] Our study did not find any significant changes in telomerase activity within the treatment group. This finding contrasts a previous study on African-American adults receiving 60,000 IU per month (equivalent to ~2000 IU per day) of vitamin D for four months, where a significant increase was observed.[20] The absence of effect may be due to the lower dose and shorter duration of vitamin D supplementation, which may not have been sufficient to influence telomerase activity, or population characteristics.

Our study found no significant changes in serum klotho levels within the treatment group. This aligns with findings showing that vitamin D can influence klotho but requires longer exposure or higher dosing.[22] Mechanistically, klotho also downregulates 1α-hydroxylase, thereby reducing active vitamin D synthesis, which may partly explain the absence of increase in this biomarker.[23, 24]

Aging-related mechanisms, including inflammation associated with cellular senescence, are often marked by increased TNF-α levels.[14,25] Our study showed no significant effect of vitamin D on TNF-α, consistent with a meta-analysis concluding that supplementation does not reliably reduce TNF-α in adults.[26] These findings suggest that TNF-α may not be a sensitive marker for assessing the anti-inflammatory effects of vitamin D supplementation.

Correlation analyses in this study, however, revealed important associations: blood glucose correlated positively with TNF-α, consistent with studies in metabolic syndrome.[27] Meanwhile cholesterol correlated negatively with TNF-α, similar to observations in hemodialysis patients.[28] Furthermore, a significant positive correlation was observed between telomerase activity and irisin, supporting prior evidence that irisin promotes telomerase function through stress pathway regulation.[29,30] These findings highlight possible molecular interactions relevant to aging biology.

This study has limitations, among others that the analysis is not adjusted for age and BMI, which are potential confounders. Vitamin D status before and after supplementation is not measured, making it difficult to confirm baseline deficiency or treatment response. In addition, the sample size is modest and the intervention period relatively short. Future studies should include larger cohorts, direct measurement of vitamin D status, and longer supplementation periods to better characterize biomarker responses.

In conclusion, vitamin D supplementation at a daily dose of 800 IU significantly increases serum irisin levels in elderly individuals, however, vitamin D supplementation has no significant effect on telomerase, klotho, or TNF-α. Correlation analyses indicate complex interactions among metabolic and inflammatory biomarkers in aging. These results suggest that vitamin D may contribute to healthy aging primarily through effects on muscle- and metabolism-related pathways rather than directly altering inflammatory or genomic stability markers

The findings support the potential role of vitamin D as an affordable and accessible strategy to promote wellness and healthy aging. By improving muscle-related biomarkers such as irisin, vitamin D supplementation may complement other lifestyle interventions—nutrition, physical activity, and metabolic control—in reducing age-related decline.

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