Original article

Role of Alphacalcidol to Reduce Pain and Serum Cartilage Oligomeric Matrix Protein in Elderly with Knee Osteoarthritis

Achmad Zaki,[1]Nurhayati Adnan Prihartono,[2]Sudarto Ronoatmodjo,[2] RatnaDjuwita,[2] SabarinahPrasetyo,[3]Andri MaruliTuaLubis,[4] Rimbawan Rimbawan,[5] Agus Hadian Rahim[6]

Abstract

Objective : To determine the effect of Alphacalcidol supplementation on pain based on WOMAC pain index and joint cartilage condition based on cartilage oligomeric matrix protein (COMP) serum markers in knee osteoarthritis (OA) elderly patients. **Materials andMethods** : This is a randomized, double-blind, and placebo-controlled trial. Symptomatic knee OA elderly subjects visited our clinic in Jakarta July-December 2017 were recruited. History taking, physical examinations, and knee radiology were performed. Serum Vitamin D (25(OH)D) and COMP were analysed using chemiluminescent immunoassay and enzyme-linked immunosorbent assay, respectively. Body mass index, physical activity, sun exposure frequency, and dietary vitamin D were assessed. Subjects were randomly allocated to either intervention group to be given 1 µg Alphacalcidol or control group to be given placebo for 12 weeks. Comparation analysis of WOMAC pain index and serum COMP concentration between both groups was performed. **Results** : There were 146 subjects participated this study. Increases of 25(OH)D were found in intervention group (2.63 ±11.24 nmol/L, p=0.05) and control groups (1.09±9.42 nmol/L, p=0.28). Alphacalcidol significantly reduced pain based on WOMAC indicator with mean reduction differences of intervention group compared to control group was 2.174±1.060 (p=0.00). COMP serum level was reduced with mean reduction differences of intervention group compared to control group was 38.15±87.553 ng/ml (p=0.39). Alphacalcidol and gender were the determinants of WOMAC pain index reduction (p=0.00 and p=0.06, respectively) while Alphacalcidol was the only serum COMP level change determinant (p=0.39). **Conclusion**s :Alphacalcidol administration reduced pain based on WOMAC indicator and COMP serum in knee OA elderly subjects.

Keywords : Vitamin D, Alphacalcidol, knee OA pain, COMP

Bangladesh Journal of Medical Science Vol. 21 No. 04 October'22 Page : 759-771
DOI: https://doi.org/10.3329/bjms.v21i4.61363

Introduction

Indonesian elderly population has been increasing in the last 30 years.[1,2] Life expectancy of Indonesian population has also been continuously increasing to 69.3 and 73.19 years old for male and female, respectively, in 2017. In 2095-2100, it is estimated to reach 82.47 and 84.97 years old for male and female, respectively.[3] Increasing elderly population potentially increases the number of degenerative diseases.

1. Department of Surgery, Faculty of Medicine, SyarifHidayatullah State Islamic University Jakarta, Jl. Kertamukti No.5 Pisangan, Ciputat, South Tangerang, Banten, Indonesia 15412
2. Department of Epidemiology, Faculty of Public Health, Universitas Indonesia, KampusBaru UI Depok, West Java, Indonesia16424
3. Biostatistics and Population Studies Department, Faculty of Public Health, Universitas Indonesia, KampusBaru UI Depok, West Java, Indonesia16424
4. Department of Orthopaedics and Traumatology, FacultyMedicine, Universitas Indonesia, CiptoMangunkusumo Hospital, Jl. Salemba Raya No. 6, Central Jakarta, Indonesia 10430
5. Community Nutrition Department, Faculty of Human Ecology, Bogor Agricultural University, Jl. Raya Dramaga, Kampus IPB Dramaga Bogor, West Java, Indonesia 16680
6. Department of Orthopaedics and Traumatology, Faculty of Medicine UniversitasPadjadjaranHasan Sadikin Hospital, Jl. Professor Eyckman No.38, Pasteur, Sukajadi, Bandung, West Java,Indonesia 40161

Correspondence: Achmad Zaki**,** Department of Surgery, Faculty of Medicine, Syarif Hidayatullah State Islamic University Jakarta. Email:achmad.zaki@uinjkt.ac.id

Osteoarthritis (OA) is the leading cause of mobility impairment. It is the fourth of the most common Years Lost to Disability (YLD) globally, which contributes to 3% of global YLD.[4] Knee OA accounted for 36,81% cases of internal medicine clinic at Manado Central Province Hospital during March 1994 – November 1995.[5] Other studies at Sanglah Denpasar Hospital in 2001-2002 showed that OA contributed to 37% of all rheumatic cases. Around 97% subjects in the study had knee OA.[5] However, national prevalence of OA in Indonesia is still not available.

Vitamin D plays important role to enhance calcium absorption in intestine and promotes mineralization of bones.[6] Several studies found that Vitamin D levels were low in patients with knee OA. Bassiouni et al found statistically lower mean serum level of vitamin D in OA patient group than in controxls (8.64 ± 6.42 vs 14.84 ± 0.87 pg/mL, p =0.0295).[7] Other study by Veronese et al showed that lower 25OHD quartiles were associated with the presence of knee OA (OR = 1.17, 95% CI 1.05–1.29).[8] Study by Jansen and Haddad showed high prevalence (around 24% of patients) low vitamin D levels in OA patients.[9]

Vitamin D deficiency were found in 50% of women age 45-55 years old and 35% of women age 60-90 years old in Jakarta and Bekasi.[10,11]Alphacalcidol is a vitamin D supplementation recommended to be used in patients with renal insufficiency, which is prevalent in elderly. Alfacalcidol does not need to undergo renal 1α-hydroxylation which impaired in renal insufficiency, although it can still cause hypercalcemia.[12,13]

Current detection and diagnosis of knee OA relies on plain radiological examination, which is not really objective because it depends on radiological expertise and experience. Moreover, plain radiological diagnosis has limited ability to detect joint damage at an early stage. Therefore, knee OA is often diagnosed at advanced stage. For this reason, selecting accurate joint cartilage biomarkers is important to detect and predict the severity of OA objectively and at earlier stage. Cartilage Oligomeric Matrix Protein (COMP) is one of degradation products released into synovium fluid and bloodstream from cartilage matrix turnover process. In a study conducted by Andriyasa and Putra, more severe knee OA tend to have higher serum COMP concentration, although there was no statistically significant correlation (r=0.127, p=0.31).[5] The CHECK (Cohort of Hip and Knee) study conducted by Van Spil et al looked at the relationship between systemic biochemical markers of joint metabolism and inflammation on radiographic parameters and pain. It was found that COMP serum as a marker of cartilage degradation was positively related to osteophyte area (stand beta - 0.092, p=0.005) but not to joint space width.[14]Another study by Bartels et al observed changes in biomarkers of bone and cartilage in knee OA patients after intensive weight loss interventions for 16 weeks. The result showed mean weight loss of 13.4 kg (95% CI: 12.5 - 14.4) and serum COMP reduction of about 1.1 (95% CI: 1.5 - 0.8) U/L which correlated to weight loss (r = -0.17, p = 0.028).[15] Therefore, COMP can be used as a marker of diagnosis and cartilage injury severity of knee OA.[8]

Currently, there has been no study about the association of Vitamin D supplementation with pain level and cartilage matrix turnover process in Indonesia. Therefore, this study aims to determine the effect of Vitamin D supplementation (Alphacalcidol) on pain and joint cartilage condition in elderly patients with knee OA.

Patientsand Methods

Study design

This is randomized, double-blind, and placebo-controlled clinical trial. The effect of Vitamin D supplementation (Alphacalcidol) on pain based on WOMAC index and on serum COMP level changes would be studied in elderly patients with knee OA. This study was started on January 2017, starting with planning and permission obtaining. The diagnosis of knee OA was made by clinical examination and x-ray. In this study, only WOMAC pain index was considered because pain is the only subjective sensations studied. In Indonesia, it is common to see elderly people came to outpatient clinic with pain as the main symptom. COMP is considered as the objective conditions of joint cartilage condition while WOMAC index is the subjective variable for detecting knee OA. Other variables that potentially affected this study were analyzed, including age, gender, body mass index (BMI), physical activity, knee radiology grade, 25(OH)D serum level, sun exposure frequency, and Vitamin D consumption. This study used serum level of 25(OH)D as the vitamin D adequacy indicator since individual vitamin D status is better reflected with 25(OH)D than its active form, $1,25(OH)_2D$ (calcitriol). The level of calcitriol is tightly regulated and maintained within its reference values even though individual actually had vitamin D deficiency.[16-18]The half-life of

25(OH)D is also longer than calcitriol, which is 2-3 weeks and 4-6 hours, respectively, making 25(OH)D a better marker of vitamin D adequacy.[17-19]This study is monitored by Contract Research Organization (CRO), The Equilab International, to ensure validity and carry out Good Clinical Practice (GCP) and Good Laboratory Practice (GLP).

Participants

Subjects with symptomatic knee OA pain who came to our clinic (Primary Health Care Clinic of UIN Jakarta) during July – December 2017 were recruited by consecutive sampling. Subjects examinations included history taking, physical examinations, knee radiology, and blood test for serum 25(OH)D, calcium, and COMP. Subjects were later divided into two groups, intervention and placebo groups.

Procedures and Variables Definitions

Serum 25(OH)D was measured using chemiluminescent immunoassay. Subjects were asked to not take any meal for 12 hours prior to 25(OH)D measurement. Serum COMP was measured using BioVendor RD194080200 Human Cartilage Oligomeric Matrix Protein Enzyme-Linked Immunosorbent Assay (ELISA). As many as 10 mL blood was obtained to measure serum COMP after at least 3 hours of sleep and 60 minutes of rest. Physical activity level was categorized using Indonesian Basic Health Research Questionnaire 2007.[20] Mild physical activity was defined as sedentary activity, including sitting, lying down, reading, playing games, or watching TV which was done for >6 hours a day. Moderate physical activity was defined as sweeping the yard, mopping, washing clothes, drawing water, planting, cleaning the bathroom or pool, playing tennis, badminton, aerobic gymnastics, swimming, basketball, soccer, or similar activity for 4-7 days a week and >60 minutes per day. Intense physical activity was defined as lifting wood, rice, sandstone, hoeing, cutting trees, fast-cycling, weightlifting, sprinting, marathons, pedicab rides, mountain climbing, or other similar activities for 4-7 days a week and >60 minutes per day. Osteoarthritis knee radiology was based on Kellgren-Lawrence knee OA grading.[21] Sun exposure was defined as ultraviolet (UV) B exposure to at least face and palms between 7.00 a.m. and 11.00 a.m. local time for 30 minutes. UVB exposure was measured using UV meter as in another study by Setiati et al.[11] Mean vitamin D consumption was assessed using Food Frequency Questionnaire (FFQ) every 4 weeks for 12 weeks. All blood test procedures were performed by certified personnel and laboratory.

Subjects were educated to have UVB exposure in face and arms area for 30 minutes at 9 AM 3 days a week without using sunblock or umbrella. Subjects were routinely contacted using telephone call by our clinic personnel every 7 days to ensure that the vitamin D supplement or placebo was taken daily and to remind the next follow up schedule. Subjects in both groups were monitored for patient adherence and side effects every two weeks in our clinic. WOMAC pain score, physical activity, sun exposure, and adverse events were monitored and reassessed. Semi quantitative Food Frequency Questionnaire (FFQ) was assessed every 4 weeks. After 12 weeks, subjects will be re-examined for changes in WOMAC pain index, knee radiography, and blood examination for 25(OH)D and COMP serum level.

Inclusion and exclusion criteria

To be eligible for becoming participant, individuals had to be around 60 to 79 years old, diagnosed as knee OA from clinical and radiological criteria, had minimum pain score of 5 (WOMAC pain index), BMI $\leq$27 kg/m^2, and were not suffering from systemic inflammation condition or disease. Using minimum WOMAC pain index score 5 inclusion criteria was done on researcher discretion because there was no standardized limit on patient inclusion based on the WOMAC pain index. Consumption of paracetamol as rescue medication was allowed in the presence of increased pain. If the pain was not reduced after oral paracetamol administration, the subject was excluded.

The exclusion criteria in this study were grade 4*Kellgren-Lawrence,* WOMAC pain index >15, serum level of 25(OH)D >125 nmol/ liter, serum calcium level >10.5 mg/dl, other kind of arthritis or inflammatory diseases, conditions that disrupt oral absorption, knee trauma history, conditions that will interact with Vitamin D, consuming supplement or steroid, undergo intraarticular therapy, and dementia. Subjects with grade 4 Kellgren-Lawrence were excluded because there was structural damage in the form of bone-on-bone contact with cartilage loss which required surgical therapy, not just limited to conservative therapy.[22,23]

Randomization and subject allocation

Subjects fulfilled inclusion and exclusion criteria were randomly allocated to intervention or placebo group using computer-generated block

permutation randomization comprising four participants per block. This procedure was performed by CRO. Group allocation was kept by CRO to ensure double-blind principle was performed. Individual subject's number of orders was informed to pharmacy depo. Individuals in intervention group were given oral Vitamin D supplements (Alphacalcidol1 µg) once daily while individuals in control group were given placebo once daily. Beside this intervention, there was no difference of any other treatment. Both groups continued the intervention for 12 weeks.

Statistical analysis

The number of subjects required was calculated using hypothesis testing for two population means by Lemeshow et al.[24] The calculation was performed twice, using both WOMAC pain score and serum COMP concentration, as separate outcome. In this study, significant mean difference of WOMAC pain score was assumed to be 2 obtained from McAlindon et al study while significant mean difference of serum COMP concentration was assumed to be 2 obtained from Forsbladd'Elia et al study.[25,26] Power assumption and confidence interval used were 80% and 95%, respectively. The number of subjects required to determine the effect of alphacalcidol supplementation on pain based on WOMAC pain scale was 68 for each arm. The number of subjects required to determine the effect of alphacalcidol supplementation on COMP serum marker was 38 for each arm. Dropout rate was estimated at 10%. Therefore, the number of subjects required for this study was 150 subjects. Data collected were analysed using SPSS for Windows 21st edition. All numerical data were presented in mean ± deviation standard. Significant differences of subjects' characteristics were determined using Chi-Square test. Comparation analysis of WOMAC pain index and serum COMP concentration between intervention group and placebo group was performed using either independent T-test or Mann-Whitney test. In the end, variables affecting WOMAC pain index and serum COMP concentration were determined using multiple linear regression.

Results

One hundred and fifty subjects participated at the start of the trial and 146 (97.3%) subjects finished the trial. Only participants completing the study were included in data and be analysed further. Subjects were recruited and followed up in July – December 2017. The subject diagram flow for this trial can be seen in figure 1 below,

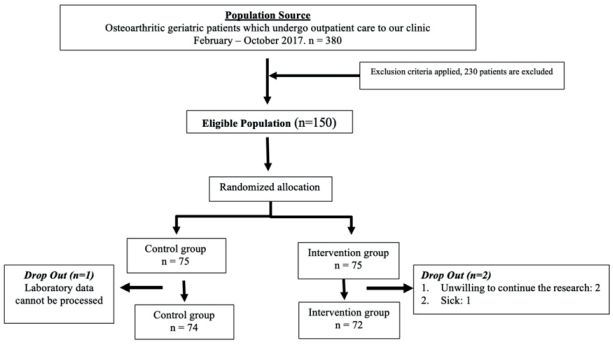

Fig 1. Subject Diagram Flow

In this study, majority of the subjects were young elderly, women, had normal BMI, mild physical activity, and had osteoarthritis grade KL 1-2. Most of the subjects were normocalcemia and exposed to sunlight ≥3times weekly. There is quite even distribution in Vitamin D consumption. The baseline characteristics are shown in table 1 below,

Table 1. Demographic characteristics. clinical status and other related factors of study subjects

Variable	Total Subjects N = 146 (%)	Intervention Group N = 72 (%)	Placebo Group N=74 (%)	*p* value
Age				
Young elderly (60-69 years old)	109 (74.7)	52 (72.2)	57 (77.0)	0.57[a]
Late Elderly (70-79 years old)	37 (25.3)	20 (27.8)	17 (23.0)	
Gender				
Man	40 (27.4)	27 (37.5)	13 (17.6)	0.01[a]
Woman	106 (72.6)	45 (62.5)	61 (82.4)	
Body Mass Index				
Severe underweight (<17 kg/m^2)	2 (1.4)	1 (1.4)	1 (1.4)	0.92[a]
Underweight (17 – 18.4 kg/m^2)	7 (4.8)	4 (5.5)	3 (4.0)	
Normal (18.5 – 25 kg/m^2)	87 (59.6)	41 (56.9)	46 (62.2)	
Overweight (25.1 – 27 kg/m^2)	50 (34.2)	26 (36.2)	24 (32.4)	
Physical Activity				
Mild	121 (82.9)	56 (77.7)	65 (87.8)	0.11[a]
Moderate	25 (17.1)	16 (22.3)	9 (12.2)	
Radiology Grade				

Variable	Total Subjects N = 146 (%)	Intervention Group N = 72 (%)	Placebo Group N=74 (%)	*p* value
Osteoarthritis KL Grade 1	61 (41.8)	30 (41.6)	31 (41.8)	0.99[a]
Osteoarthritis KL Grade 2	59 (40.4)	29 (40.3)	30 (40.5)	
Osteoarthritis KL Grade 3	26 (17.8)	13 (18.1)	13 (17.7)	
Serum 25(OH)D Level				
Sufficient (>50 – 125 nmol/l)	50 (34.3)	29 (40.2)	21 (28.3)	0.19[a]
Insufficient (25 – 50 nmol/l)	78 (53.4)	33 (46.0)	45 (62.5)	
Deficient (<25 nmol/l)	18 (12.3)	10 (13.8)	8 (9.2)	
Serum Calcium Level				
Normocalcemia (8.5-10.5 mg/dl)	138 (94.5)	66 (91.7)	72 (97.3)	0.163[a]
Hypocalcemia (<8.5 mg/dl)	8 (5.5)	6 (8.3)	2 (2.7)	
Sun Exposure Frequency				
< 3 times a week	46 (31.5)	21 (29.2)	25 (33.8)	0.6[a]
≥ 3 times a week	100 (68.5)	51 (70.8)	49 (66.2)	
Vitamin D Consumption				
Quartile 1 (0.00-1.10 mcg/day)	39 (26.7)	19 (26.4)	20 (27.0)	0.99[a]
Quartile 2 (1.11-2.03 mcg/day)	35 (24.0)	17 (23.6)	18 (24.3)	
Quartile 3 (2.04 – 3.35 mcg/day)	37 (25.3)	18 (25.0)	19 (25.7)	
Quartile 4 (>3.35 mcg/day)	35 (24.0)	18 (25.0)	17 (23.0)	
WOMAC Pain Index	7.9[b] 7[c] (5-15)[d]	8[b]	7.8[b]	0.63[e]
Serum COMP Level (ng/ml)	743.9[b] 684.5[c] (355-2160)[d]	732.99[b]	754.52[b]	0.94[e]

a:Chi-Square test ;b:Mean ; c:Median ; d: Min – Max ; e: Mann-Whitney test

Mean serum 25(OH)D level were observed to be increased in both groups. Significant increase (2.63 ±11.24 nmol/L) was found in intervention group, from 45.92 ±17.64 nmol/L (week 0) to 48.55±17.16 nmol/L (week 12) (p=0.05). In placebo group, 25(OH)D serum levels was increased 1,09±9,42 nmol/L, from 1.99±16.09 (week 0) to 43.09±15.71 (week 12) (p=0.28). When increased serum 25(OH)D level in both groups were compared, no statistically significant different was observed (p=0.24).

WOMAC pain index was observed to be reduced in both groups. In intervention group, mean WOMAC pain index was reduced 7.04± 0.56, from 8±2.30 to 0.96 ±1.56. Pain reduction was also observed in placebo group for 5.09 ± 0.88, from 7.8 ±2.14 to 2.70 ±3.03. When mean WOMAC pain index reduction in both groups were compared, there was statistically significant difference (p=0.001). Mean difference of WOMAC pain index between week 0 and week 12 in both intervention and placebo group according to several variables could be seen in table 2,

Vitamin D administration changed serum COMP level. In intervention group, mean difference serum COMP level was -4.66 ± 61.40 ng/mL, from 732.99 ± 266.28 ng/mL to 728.33 ± 319.22 ng/mL. However, serum COMP level was found to be increased in placebo group for 33.49 ± 63.39 ng/mL, from 754.52 ± 328.53 ng/mL to 788.01 ± 350.49 ng/mL. When mean difference of serum COMP level in both groups were compared, there was no statistically significant difference (p=0.39). Mean differences of serum COMP level between week 0 and week 12 in both intervention and placebo group according to several variables could be seen in table 3 below,

Multivariate analysis using multiple linier regression was performed to determine variables that affect WOMAC pain index and serum COMP changes between week 0 and week 12 in both intervention group and placebo group. The main independent variable was Vitamin D supplementation (*Alphacalcidol*). Covariate variables in early model were age, gender, BMI, mean physical activity in 12 weeks of trial, knee OA severity radiology grade, concentration difference of 25(OH)D serum concentration before and after intervention, mean sun exposure frequency in 12 weeks, and mean Vitamin D consumption in 12 weeks. Final model analysis found all assumption of multiple linear regressionwere fulfilled.

Vitamin D supplementation (Alphacalcidol) and gender were the determinant variables of WOMAC pain index reduction (Table 4). Interaction between gender and Vitamin D supplementation variable was not found (p>0.05). Vitamin D supplementation variable was the only determinant variables of serum COMP level changes but not statistically significant (p=0.39) (Table 5). During this study recruitment and follow up period, no subjects experienced any adverse event nor side effects.

Table 2. Mean Difference of Knee OA Pain Based on WOMAC Pain Index inIntervention Group and Placebo Group Categorized into Several Dependent Variables

Dependent Variables	Intervention Group		Placebo Group		Mean Differences Between Intervention and Placebo Groups	*p* value
	n	Mean Difference Before and After Intervention (± SD)	n	Mean Difference Before and After Intervention (± SD)	Mean (95% CI)	
Age						
Young Elderly (60-69 yo)	52	-6.96 (±2.41)	57	-5.21 (±3.86)	-1.75 (-2.98 . (-0.51))	0.005[a]
Old Ederly (70-79 yo)	20	-7.25 (±2.40)	17	-4.71 (±3.67)	-2.54 (-4.59 . (-0.50))	0.02[a]
Gender						
Man	27	-6.41 (± 2.37)	13	-4.00 (± 3.53)	-2.41 (-4.32 . (-0.49))	0.08[a]
Woman	45	-7.42 (± 2.35)	61	-5.33 (± 3.84)	-1.77 (-3.29 . (-0.89))	0.002[a]
Body Mass Index (kg/m2)						
Underweight (< 18.5 kg/m^2)	5	-7.2(± 2.59)	4	-9.25 (± 3.59)	2.05 (-2.80 . 6.90)	0.35[b]
Normal(18.5 -25 kg/m^2)	41	-6.85 (± 2.17)	46	-5.00 (± 3.75)	-1.85 (-3.15. (-0.56))	0.01[a]
Overweight(25.1-27 kg/m^2)	26	-7.31 (± 2.74)	24	-7.31 (± 2.74)	-2.72 (-4.55 . (-0.89))	0.01[a]
Physical Activity						
Mild physical activity	68	-7.13 (± 2.42)	73	-5.08 (± 3.82)	-2.05 (-3.11. (-0.99))	0.00[a]
Moderate physical activity	4	-5.5 (± 1.29)	1	-6.0	-0.5 (-4.09.5.09)	0.08[b]
Knee Radiology Grade						
OA KL grade 1	30	-7.03 (± 2.62)	31	-4.9 (± 4.25)	-2.13 (-3.95 . (-0.31))	0.04[a]
OA KL grade 2	29	-7.00 (± 2.19)	30	-4.8 (± 3.54)	-2.20 (-3.74 . (-0.66))	0.01[a]
OA KL grade 3	13	-7.15 (± 2.48)	13	-6.23 (± 3.24)	-0.92 (-3.26 . 1.41)	0.55[a]
Serum 25(OH)D Level						
Sufficient (>50-125 nmol/L)	34	-6.56 (± 2.30)	24	-5.37 (± 3.80)	-1.18 (-2.95. 0.58)	0.19[a]
Insufficient (25-50 nmol/L)	33	-7.42 (± 2.28)	39	-4.87 (± 4.17)	-2.55 (-4.11. (-0.99))	0.005[a]
Deficient (<25 nmol/L)	5	-7.80 (± 3.56)	11	-5.27 (± 2.33)	-2.53 (-5.7. 0.64)	0.11[b]
Sun Exposure Frequency						
<3 times/week	29	-6.89 (± 2.09)	37	-4.89 (± 3.71)	-2.01 (-3.45. (-0.56))	0.02[a]
≥ 3 times/week	43	-7.14 (±2.59)	37	-5.30(±3.92)	-1.84 (-3.30. (-0.38))	0.02[a]
Vitamin D Consumption						
Quartile 1 (0.00-1.10 mcg/day)	11	-8.54 (± 3.08)	11	-5.54 (± 5.39)	-3.00 (-6.90. 0.90)	0.13[b]
Quartile 2 (1.11-2.03 mcg/day)	21	-7.28 (± 0.46)	22	-4.91 (± 0.86)	-2.38 (-4.37. (-0.04))	0.04[a]
Quartile 3 (2.04 – 3.35 mcg/day)	19	-6.47 (± 2.24)	18	-4.83 (± 3.74)	-1.64 (-3.68. 0.41)	0.11[b]
Quartile 4 (>3.35 mcg/day)	21	-6.52 (± 2.18)	23	-5.26 (± 2.81)	-1.27 (-2.81. 0.28)	0.6[a]
Combined Variables	72	-7.04 (± 0.56)	74	-5.09 (±0.88)	-1.95 (-2.99. -0.91)	0.001[a]

[a]: Mann-Whitney Test

[b]: Independent T-Test

Table 3. Mean Difference ofJoint Cartilago Changes Based on Serum COMP Level in Intervention Group and Placebo GroupCategorized into Several Dependent Variables

Dependent Variable	Intervention Group		Placebo Group		Mean Difference between Intervention and Placebo Groups	*p* value
	n	Mean Difference Before and After Intervention (± SD)	n	Mean Difference Before and After Intervention (± SD)	Mean (95% CI)	
Age (years old)						
Young Elderly (60-69)	52	-3.29 (± 244.97)	57	27.96 (±225.44)	-31.25 (-120.56 . 58.08)	0.39[a]
Old Ederly (70-79)	20	-8.21 (±306.69)	17	52.05 (±403.85)	-60.26 (-297.84 . 177.09)	0.61[b]
Gender						
Man	27	28.91 (± 254.31)	13	13.45 (± 416.71)	15.46 (-199.65 . 230.59)	0.89[b]
Woman	45	-24.8 (±266.19)	61	37.77 (±237.11)	-62.57 (-159.92 . 34.79)	0.21[b]
Body Mass Index (kg/m²)						
Underweight (< 18.5)	5	44.60 (± 434.77)	4	65.42 (± 256.63)	-20.82 (-606.31 . 564.66)	1.00[a]
Normal(18.5 -25)	41	-3.86 (± 265.16)	46	49.27 (± 242.93)	-53.13 (-161.45 . 55.17)	0.33[b]
Overweight(25.1-27)	26	-15.37 (± 224.88)	24	-2.08 (± 333.91)	-13.29 (-174.03 . 147.45)	0.87[b]
Physical Activity						
Mild physical activity	68	-3.76 (± 262.97)	73	32.64 (± 274.99)	-35.40 (-125.13. 54.32)	0.44[b]
Moderate physical activity	4	-19.82 (± 266.89)	1	168.8	-188.63(-1138.26 . 761.01)	0.40[a]
Knee Radiology Grade						
OA KL grade 1	30	61.16 (± 272.07)	31	2.15 (± 291.83)	59.01 (-65.10 . 183.13)	0.34[b]
OA KL grade 2	29	-23.15 (± 269.09)	30	61.98 (± 273.54)	-85.13 (-226.63 . 56.38)	0.21[a]
OA KL grade 3	13	-115.28 (± 176.76)	13	42.50 (± 400.38)	-157.78 (-408.31 . 92.74)	0.21[a]
25(OH)D Serum Level (nmol/L)						
Sufficient (>50-125)	34	-52.44 (± 217.87)	24	4.71 (± 343.69)	-47.73 (-199.28.179.89)	0.52[b]
Insufficient (25-50)	33	58.55 (± 296.72)	39	54.23 (± 254.44)	4.32 (-125.21.133.84)	0.78[a]
Deficient (<25)	5	-98.68 (± 230.27)	11	43.31 (± 152.84)	-140.19 (-1346.59.66.22)	0.16[b]
Sun exposure frequency						
<3 times/week	29	-17.04 (± 274.52)	37	39.86 (± 249.31)	-56.91 (-186.05.72.23)	0.34[a]
≥ 3 times/week	43	3.70 (± 54.97)	37	27.12 (± 49.18)	-23.42 (-146.75.99.91)	0.71[b]
Vitamin D Consumption (mcg/day)						
Quartile 1 (0.00-1.10)	11	-90.46(± 247.26)	11	-54.67 (± 446.59)	-35.79 (-365.41.293.03)	0.82[b]
Quartile 2 (1.11-2.03)	21	34.79 (± 336.25)	22	100.28 (± 271.32)	-65.49 (-253.24.122.25)	0.48[b]
Quartile 3 (2.04 – 3.35)	19	-29.49 (± 201.63)	18	-24.42 (± 227.14)	-5.07 (-148.23.138.09)	0.94[b]
Quartile 4 (>3.35)	21	23.32 (± 219.19)	23	57.09 (± 189.00)	-33.78 (-158.11.90.56)	0.84[b]
Combined Variables	72	-4.66 (± 61.40)	74	33.49 (± 63.38)	-38.15 (-125.7 . 49.41)	0.39[b]

[a]: Mann-Whitney Test

[b]: Independent T-Test

Table 3. Mean Difference ofJoint Cartilago Changes Based on Serum COMP Level in Intervention Group and Placebo GroupCategorized into Several Dependent Variables

Dependent Variable	Intervention Group		Placebo Group		Mean Difference between Intervention and Placebo Groups	*p* value
	n	Mean Difference Before and After Intervention (± SD)	n	Mean Difference Before and After Intervention (± SD)	Mean (95% CI)	
Age (years old)						
Young Elderly (60-69)	52	-3.29 (± 244.97)	57	27.96 (±225.44)	-31.25 (-120.56 . 58.08)	0.39[a]
Old Ederly (70-79)	20	-8.21 (±306.69)	17	52.05 (±403.85)	-60.26 (-297.84 . 177.09)	0.61[b]
Gender						
Man	27	28.91 (± 254.31)	13	13.45 (± 416.71)	15.46 (-199.65 . 230.59)	0.89[b]
Woman	45	-24.8 (±266.19)	61	37.77 (±237.11)	-62.57 (-159.92 . 34.79)	0.21[b]
Body Mass Index (kg/m^2)						
Underweight (< 18.5)	5	44.60 (± 434.77)	4	65.42 (± 256.63)	-20.82 (-606.31 . 564.66)	1.00[a]
Normal(18.5 -25)	41	-3.86 (± 265.16)	46	49.27 (± 242.93)	-53.13 (-161.45 . 55.17)	0.33[b]
Overweight(25.1-27)	26	-15.37 (± 224.88)	24	-2.08 (± 333.91)	-13.29 (-174.03 . 147.45)	0.87[b]
Physical Activity						
Mild physical activity	68	-3.76 (± 262.97)	73	32.64 (± 274.99)	-35.40 (-125.13. 54.32)	0.44[b]
Moderate physical activity	4	-19.82 (± 266.89)	1	168.8	-188.63(-1138.26 . 761.01)	0.40[a]
Knee Radiology Grade						
OA KL grade 1	30	61.16 (± 272.07)	31	2.15 (± 291.83)	59.01 (-65.10 . 183.13)	0.34[b]
OA KL grade 2	29	-23.15 (± 269.09)	30	61.98 (± 273.54)	-85.13 (-226.63 . 56.38)	0.21[a]
OA KL grade 3	13	-115.28 (± 176.76)	13	42.50 (± 400.38)	-157.78 (-408.31 . 92.74)	0.21[a]
25(OH)D Serum Level (nmol/L)						
Sufficient (>50-125)	34	-52.44 (± 217.87)	24	4.71 (± 343.69)	-47.73 (-199.28.179.89)	0.52[b]
Insufficient (25-50)	33	58.55 (± 296.72)	39	54.23 (± 254.44)	4.32 (-125.21.133.84)	0.78[a]
Deficient (<25)	5	-98.68 (± 230.27)	11	43.31 (± 152.84)	-140.19 (-1346.59.66.22)	0.16[b]
Sun exposure frequency						
<3 times/week	29	-17.04 (± 274.52)	37	39.86 (± 249.31)	-56.91 (-186.05.72.23)	0.34[a]
≥ 3 times/week	43	3.70 (± 54.97)	37	27.12 (± 49.18)	-23.42 (-146.75.99.91)	0.71[b]
Vitamin D Consumption (mcg/day)						
Quartile 1 (0.00-1.10)	11	-90.46(± 247.26)	11	-54.67 (± 446.59)	-35.79 (-365.41.293.03)	0.82[b]
Quartile 2 (1.11-2.03)	21	34.79 (± 336.25)	22	100.28 (± 271.32)	-65.49 (-253.24.122.25)	0.48[b]
Quartile 3 (2.04 – 3.35)	19	-29.49 (± 201.63)	18	-24.42 (± 227.14)	-5.07 (-148.23.138.09)	0.94[b]
Quartile 4 (>3.35)	21	23.32 (± 219.19)	23	57.09 (± 189.00)	-33.78 (-158.11.90.56)	0.84[b]
Combined Variables	72	-4.66 (± 61.40)	74	33.49 (± 63.38)	-38.15 (-125.7 . 49.41)	0.39[b]

[a]: Mann-Whitney Test

[b]: Independent T-Test

Table 4. Determinants of Knee Pain Score Changes

Variables	Full Model of Knee Pain Score Change Determinants			Final Model of Knee Pain Score Change Determinants		
	B coefficient	95% CI	*p* value	B coefficient	95% CI	*p* value
Vitamin D Supplementation (Alphacalcidol)	-2.119	-3.201 , (-1.037)	0.000	-2.17	-3.233 , (-1.114)	0.00
Gender	-1.106	-2.369 , 0.157	0.086	-1.14	-2,324 , 0,51	0.06
Age	0.059	-1.244 , 1.363	0.929			
BMI	0.558	-0.374 , 1.491	0.238			
Physical activity	0.355	-2.631 , 3.342	0.814			
Knee radiology grade	0.235	-0.516 , 0.986	0.537			
25(OH)D serum level	-0.021	-0.072 , 0.030	0.423			
Sun exposure frequency	-0.397	-1.455 , 0.661	0.459			
Vitamin D consumption	0.276	-0.783 , 0.231	0.284			

Table 5. Determinants of Serum COMP Changes

Variables	Full Model of Serum COMP Change Determinants			Final Model of Serum COMP Change Determinants		
	B coefficient	95% CI	*p* value	B coefficient	95% CI	*p* value
Vitamin D Supplementation (Alphacalcidol)	-39.331	-132.19 , 53.532	0.404	-38.15	-125.703 , 49.409	0.39
Gender	-12.344	-120.69 , 96.012	0.822			
Age	16.849	-94.995 , 128.69	0.766			
BMI	26.054	-53.979 , 106.08	0.521			
Physical activity	8.803	-247.44 , 265.05	0.946			
Knee radiology grade	26.034	-38.439 , 90.508	0.426			
25(OH)D serum level	-0.852	-5.228 , 3.525	0.701			
Sun exposure frequency	-1.087	-91.869 , 89.694	0.981			
Vitamin D consumption	-13.743	-57.272 , 29.876	0.533			

Discussion

Elderly population is susceptible to Vitamin D insufficiency, deficiency, and chronic musculoskeletal pain. A systematic review by Chapple et al found strong evidence that age was a predictor of knee OA progression.[27]In this study, majority subjects (53.4%) had insufficient 25(OH)D levels (25-50 nmol/L). Moreover, 12.3% subjects had Vitamin D deficiency (<25 nmol/L). The prevalence is fewer compared to clinical studies by Setiati et al which found 35.1% Indonesian elderly women had Vitamin D deficiency.[11] However, the results of Setiati et al study may be caused by wider range of subjects' age involved (60-90 years). Prevalence of Vitamin D deficiency is also higher in Afrin et al study at Bangladesh (43.8%), with also wider spectrum of age group.[28]There is a tendency that serum 25(OH)D level is lower as subject gets older.

In this study, alphacalcidol was used because it does not need to undergo 1a-hydroxylation process, which is impaired in patients with renal insufficiency.[12,13] In addition, the effects of hypercalcemia are not higher than other vitamin D supplementations.[13] However, hypercalcemia effect on taking alphacalcidol supplementation cannot be ignored. Therefore, this study excluded subjects with high 25(OH)D levels.

This study found a significant increase of 25(OH) D serum level after alphacalcidol supplementation. This finding is interesting since previous studies reported increase of 25(OH)D serum level after native

vitamin D, not alfacalcidol, supplementation. Results obtained from a study by Jin et al which showed an average increase in 25(OH)D serum level of 40.6 nmol/L in intervention group (50,000 IU/month Vitamin D_3 supplementation for 2 years) compared to an increase of only 6.7 nmol/L in control group.[28] Greater increases in 25(OH)D serum level could be due to Vitamin D supplementation at larger and longer doses. Other study by Yosephin et al, which used Vitamin D supplementation of 400 IU for 12 weeks in reproductive age women workers in Bogor, showed 6.3 ng/dL (baseline 14.9 ng/dL) or 42.3% increase in serum 25(OH)D levels.[29]Although the mechanism of how alphacalcidol supplementation increase 25(OH)D is unclear, it is possible that alphacalcidol is converted to $1,25(OH)_2D$ (calcitriol) and exerts negative feedback to 1α-hydroxylation process by downregulating 1a-hydroxilase. Downregulated 1α-hydroxylase makes 25(OH)D stays in its form and is not converted to calcitriol.[30]

This study shows that there is a significant decrease in the mean score of WOMAC indicator pain levels in the intervention group. The difference in the reduction in OA pain levels between the intervention group and the control group is statistically significant as well. Clinically, This result is better than Schlogl et al who reported differences of WOMAC pain level between intervention group and placebo after Vitamin D supplementation was -0.77 (p=0.04).[31] This difference in results can be influenced by the dose of Vitamin D supplementation given.

Age, gender, body mass index, KL grade, initial serum 25(OH)D level, sunlight exposure, and vitamin D supplementation play role in influencing WOMAC pain index level as showed by several studies.[32-40] Multiple linear regression analysis results that Vitamin D (Alphacalcidol) supplementation for 12 weeks influences the reduction in pain level based on WOMAC indicator. The results of the contents are in accordance with the study of Sanghi et al which showed improvement of pain level in Vitamin D supplementation-administered group for 12 months.[41] This study also complements metanalysis by Wu et al which observe the effect of Vitamin D supplementation on changes in the degree of chronic pain.[42] The study results in a significant pain reduction in intervention group compared to placebo.

Vitamin D has been proven to stimulate proteoglycan synthesis in joint cartilage in vitro.[43] In this study, serum COMP levels reduction was observed in intervention group. Meanwhile in control group, serum COMP level was increased. No statistically significant difference in mean differences between serum COMP levels of pre and post-intervention interventions between both groups. It can be caused by the amount of dose and duration of Vitamin D supplementation that is not large and long enough which cannot produce a statistically significant difference yet.

No statistically significant serum COMP levels change was observed across several variables as shown in Table 3. It supports several studies published previously.[44-47] Despite that, studies related to COMP and the variables until this time show contradictive results. There are no studies relating Vitamin D level with serum COMP level change in knee OA up until this study was done. However, the results obtained in final model showed that supplementation of Vitamin D (Alphacalcidol) for 12 weeks contributed to the improvement of the knee joint cartilage as indicated by a decrease in serum COMP level after intervention.

This is the first study of knee OA in Indonesia which combines symptoms modifier indicator (WOMAC pain indicator) with disease modifier indicator (joint cartilage damage). Therefore, the protocol of this study was designed by authors. There are several limitations of this study. The first limitation is difficulty in objectively assessing the degree of pain becomes a potential information bias. Information bias may also occur in the process of filling semi-quantitative FFQ. However, the authors had put efforts in maintaining the originality and acceptability of the result of this process by training the interviewers and informing subjects about this process. In addition, measurements of COMP concentrations carried out through blood serum collection may be influenced by the presence of non-clinical OA or RA disease processes in respondents.

Conclusion

Vitamin D supplementation (Alphacalcidol) for 12 weeks affects the reduction of pain level based on WOMAC indicators and the reduction of serum COMP levels in elderly with knee OA although the reduction of serum COMP was not statistically significant. Vitamin D supplementation can benefit the elderly with early knee OA and Vitamin D insufficiency to reduce the pain of the knee joint.

Ethics Approval

The procedures followed were in accordance with the ethical standards of the responsible committee on

human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards. This study was approved by the Institutional Review Board Faculty of Public Health Universitas Indonesia No. 162/UN2.F10/PPM.00.02/2017

Conflict of Interest Statement

None declared

Funding

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

Conflict of Interest

All authors declare that they have no conflict of interest.

Trial Registration Number

This clinical study was registered in clinicaltrials.gov with ID NCT04405960

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Acknowledgements

The authors would like to appreciate Teijin Pharma Limited Japan for alphacalcidol 1 mcg donation without any binding contract.

Authors Contributions

Achmad Zaki made substantial contributions in concepting, designing, analyzing, interpreting, and drafting the study.Nurhayati Adnan Prihartono, SudartoRonoatmodjo, RatnaDjuwita, SabarinahPrasetyo, Andri MT Lubis, Rimbawan, and AgusHadian Rahim made substantial contributions in concepting, drafting, and revising the study. All authors have read and approved the final version of this study.

References

1. Statistik penduduk lanjut usia 2017. Jakarta: Statistics Indonesia; 2017.
2. Statistics Indonesia. Survei sosial ekonomi nasional 2013 [Internet]. Jakarta: Statistics Indonesia; 2013. Available from: https://microdata.bps.go.id/mikrodata/index.php/catalog/220
3. United Nations Department of Social and Economic Affairs. World population prospects: The 2012 revision. Geneva: Population Division; 2013.
4. Symmons D, Mathers C, Pfleger B. Global burden of osteoarthritis in the year 2000 [Internet]. Geneva: World Health Organization; 2000. Available from: https://www.who.int/healthinfo/statistics/bod_osteoarthritis.pdf
5. Andriyasa K, Putra TR. Korelasi antara derajat beratnya osteoarthritis lutut dan cartilage oligomeric matrix protein serum. *J Intern Med*. 2012;**13**(1):10–20.
6. Gueli N, Verrusio W, Linguanti A, Di Maio F, Martinez A, Marigliano B, et al. Vitamin D: drug of the future. A new therapeutic approach. *Archives of Gerontology and Geriatrics*. 2012;**54**(1):222–7.
7. Bassiouni H, Aly H, Zaky K, Abaza N, Bardin T. Probing the relation between vitamin D deficiency and progression of medial femoro-tibial osteoarthitis of the knee. *Curr Rheumatol Rev*.2017;**13**(1):65–71.
8. Verma P, Dalal K. Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: A novel diagnostic and prognostic biomarker. *J Orthop Res*. 2013;**31**(7):999–1006.
9. Jansen J, Haddad F. High prevalence of vitamin D deficiency in elderly patients with advanced osteoarthritis scheduled for total knee replacement associated with poorer preoperative functional state. *Ann R Coll Surg Engl*. 2013;**95**(8):569–72.
10. Oemardi M, Horowitz M, Wishart JM, Morris HA, Need AG, O?Loughlin PD, et al. The effect of menopause on bone mineral density and bone-related biochemical variables in Indonesian women. *Clin Endocrinol*. 2007;**67**(1):93–100.
11. Setiati S. Pengaruh pajanan sinar ultraviolet B bersumber dari sinar matahari terhadap konsentrasi vitamin D (25(OH)D) dan hormon paratiroit pada perempuan usia lanjut Indonesia. *Nat Public Health J*. 2008;**2**(4):147.
12. Ebeling PR, Adler RA, Jones G, Liberman UA, Mazziotti G, Minisola S, et al. Management of endocrine disease: Therapeutics of vitamin D. *Eur J Endocrinol*. 2018;R239–59.
13. Palmer SC, McGregor DO, Craig JC, Elder G, Macaskill P, Strippoli GF. Vitamin D compounds for people with chronic kidney disease requiring dialysis. Cochrane Kidney and Transplant Group, editor. Cochrane Database Sys Rev [Internet]. 2009 Oct 7 [cited 2020 Apr 15]; Available from: http://doi.wiley.com/10.1002/14651858.CD005633.pub2
14. Schiphof D, Runhaar J, Waarsing JH, van Spil WE, van Middelkoop M, Bierma-Zeinstra SMA. The clinical and radiographic course of early knee and hip osteoarthritis over 10 years in CHECK (Cohort Hip and Cohort Knee). *Osteoarthritis Cartilage*. 2019;**27**(10):1491–500.
15. Bartels EM, Christensen R, Christensen P, Henriksen M, Bennett A, Gudbergsen H, et al. Effect of a 16 weeks weight loss program on osteoarthritis biomarkers in obese patients with knee osteoarthritis: a prospective cohort study. *Osteoarthritis Cartilage*. 2014;**22**(11):1817–25.
16. Dirks N, Ackermans M, Lips P, de Jongh R, Vervloet M, de Jonge R, et al. The When, What & How of Measuring Vitamin D Metabolism in Clinical Medicine. *Nutrients*. 2018;**10**(4):482.
17. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*. 2006;**81**(3):353–73.
18. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. *Ann Epidemiol*. 2009;**19**(2):73–8.
19. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. *J Clin Endocrinol Metab*. 2011;**96**(1):53–8.
20. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI. Basic Health Research (Riset Kesehatan Dasar) 2007 [Internet]. Kementerian Kesehatan RI; 2007. Available from: http://labdata.litbang.kemkes.go.id/images/download/laporan/RKD/2007/Riskesdas_2007_English.zip
21. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*.1957;**16**(4):494–502.
22. Guermazi A, Hayashi D, Roemer F, Felson DT, Wang K, Lynch J, et al. Severe radiographic knee osteoarthritis – does Kellgren and Lawrence grade 4 represent end stage disease? – the MOST study. *Osteoarthritis Cartilage*. 2015;**23**(9):1499–505.
23. Katz JN, Earp BE, Gomoll AH. Surgical management of osteoarthritis. *Arthritis Care Res*. 2010;**62**(9):1220–8.
24. Lemeshow S. Adequacy of sample size in health studies. World Health Organization, editor. New York, USA: World Health Organization; 1990. 239 p.
25. McAlindon T, LaValley M, Schneider E, Nuite M, Lee JY, Price LL, et al. Effect of Vitamin D supplementation on progression of knee pain and cartilage volume loss in

patients with symptomatic osteoarthritis: A randomized controlled trial. *JAMA*. 2013;**309**(2):155.

26. Forsblad d'Elia H, Christgau S, Mattsson L-A, Saxne T, Ohlsson C, Nordborg E, et al. Hormone replacement therapy, calcium and vitamin D3 versus calcium and vitamin D3 alone decreases markers of cartilage and bone metabolism in rheumatoid arthritis: a randomized controlled trial [ISRCTN46523456]. *Arthritis Res Ther*. 2004;**6**(5):R457-468.
27. Chapple CM, Nicholson H, Baxter GD, Abbott JH. Patient characteristics that predict progression of knee osteoarthritis: A systematic review of prognostic studies. *Arthritis Care Res*. 2011;**63**(8):1115–25.
28. Afrin SF, Rahman MH, Bari KF, Al-Mahmood AKA. Status of Vitamin D by measuring 25 - Hydroxyvitamin D level in Dhaka City. *Bangladesh Journal of Medical Science*. 2019 ;**18**(03) : 624-627
29. Jin X, Jones G, Cicuttini F, Wluka A, Zhu Z, Han W, et al. Effect of vitamin D supplementation on tibial cartilage volume and knee pain among patients with symptomatic knee osteoarthritis: A randomized clinical trial. *JAMA*. 2016;**315**(10):1005.
30. Yosephin B, Khomsan A, Briawan D, Rimbawan R. Vitamin D plus calcium supplementation increased serum 25(OH)D on reproductive age women workers. *Asian Pac J Trop Dis*. 2015;**5**(11):877–80.
31. Ringe JD, Faber H, Fahramand P, Schacht E. Alfacalcidol versus plain vitamin D in the treatment of glucocorticoid/ inflammation-induced osteoporosis. *J Rheumatol Suppl*. 2005;**76**:33–40.
32. Schlögl M, Chocano-Bedoya P, Dawson-Hughes B, Orav EJ, Freystaetter G, Theiler R, et al. Effect of monthly Vitamin D on chronic pain among community-dwelling seniors: A randomized, double-blind controlled trial. *J Am Med Dir Assoc*. 2019;**20**(3):356–61.
33. Andersen RE, Crespo CJ, Ling SM, Bathon JM, Bartlett SJ. Prevalence of significant knee pain among older Americans: Results from the third national health and nutrition examination survey. *J Am Geriatr Soc*. 1999;**47**(12):1435–8.
34. Bennell KL, Hunter DJ, Hinman RS. Management of osteoarthritis of the knee. *BMJ*. 2012;345:e4934.
35. Ding C, Cicuttini F, Parameswaran V, Burgess J, Quinn S, Jones G. Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: The Tasmanian older adult cohort study. *Arthritis Rheum*. 2009;**60**(5):1381–9.
36. Jungert A, Spinneker A, Nagel A, Neuhäuser-Berthold M. Dietary intake and main food sources of vitamin D as a function of age, sex, vitamin D status, body composition, and income in an elderly German cohort. *Food Nutr Res*. 2014;**58**(1):23632.
37. Muraki S, Oka H, Akune T, Mabuchi A, En-yo Y, Yoshida M, et al. Prevalence of radiographic knee osteoarthritis and its association with knee pain in the elderly of Japanese population-based cohorts: The ROAD study. *Osteoarthritis Cartilage*. 2009;**17**(9):1137–43.
38. Muraki S, Dennison E, Jameson K, Boucher BJ, Akune T, Yoshimura N, et al. Association of vitamin D status with knee pain and radiographic knee osteoarthritis. *Osteoarthritis Cartilage*. 2011;**19**(11):1301–6.
39. Salehpour A, Hosseinpanah F, Shidfar F, Vafa M, Razaghi M, Dehghani S, et al. A 12-week double-blind randomized clinical trial of vitamin D3 supplementation on body fat mass in healthy overweight and obese women. *Nutr J*. 2012;**11**(1):78.
40. Vanlint S. Vitamin D and obesity. *Nutrients*. 2013;**5**(3):949–56.
41. Mavis ME, Yildrimmavis C, Arancioglu IO, Yilmaz HK, Erguden B, Hatemi HH.Evaluation of vitamin D levels and body mass indexes of university employees. *Bangladesh Journal of Medical Science.* 2020;**19**(2):*229-236*
42. Sanghi D, Mishra A, Sharma AC, Singh A, Natu SM, Agarwal S, et al. Does Vitamin D improve osteoarthritis of the knee: A randomized controlled pilot trial. *Clin Orthop Relat Res*. 2013;**471**(11):3556–62.
43. Wu Z, Malihi Z, Stewart AW, Lawes CM, Scragg R. Effect of Vitamin D supplementation on pain: A systematic review and meta-analysis. *Pain Physician*. 2016;**19**(7):415–27.
44. Corvol MT, Dumontier MF, Tsagris L, Lang F, Bourguignon J. Cartilage and vitamin D in vitro. *Ann Endocrinol* (Paris).1981;**42**(4–5):482–7.
45. Andersson ML, Thorstensson CA, Roos EM, Petersson IF, Heinegård D, Saxne T. Serum levels of Cartilage Oligomeric Matrix Protein (COMP) increase temporarily after physical exercise in patients with knee osteoarthritis. *BMC Musculoskelet Disord*. 2006;**7**(1):98.

46 Clark AG, Jordan JM, Vilim V, Renner JB, Dragomir AD, Luta G, et al. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County Osteoarthritis Project. *Arthritis Rheum*. 1999;**42**(11):2356–64.

47. Wisłowska M, Jabłońska B. Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis and knee osteoarthritis. *Clin Rheumatol*. 2005;**24**(3):278–84.