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Relationship of serum magnesium and vitamin D levels to range of motion and 30-second sit to stand test of knee osteoarthritis patients

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ABSTRACT

Background. Knee osteoarthritis (OA) is a prevalent degenerative joint disease and a leading cause of disability. Limited range of motion (ROM) and impaired physical performance significantly affect daily activities. Current assessment methods rely on radiography, emphasizing the need for identifying modifiable risk factors such as micronutrients. Magnesium (Mg) and vitamin D play a critical role in joint, bone, and muscle function. This study evaluates the relationship between serum Mg and vitamin D levels with ROM and the 30-second sit-to-stand (30STS) test.

Methods. This cross-sectional study included 44 patients with primary knee OA. Serum Mg and vitamin D levels were analyzed for their association with ROM and 30STS test using the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis determined optimal cut-off points for distinguishing normal and abnormal ROM and 30STS performance.

Results. The average Mg level was found to be within normal limits (1.91 mmol/L), while vitamin D levels were in the insufficient category (17.56 ng/mL). The results of the analysis showed that Mg levels did not show significant differences to ROM ($p> 0.05$) and 30STS ($p> 0.05$) while vitamin D levels showed significant differences to ROM ($p<0.05$) and 30STS ($p<0.05$).

Conclusion. There was a significant relationship between serum vitamin D levels to ROM and 30STS, however, no significant relationship was found between Mg levels and both parameters in knee OA patients. These findings highlight the potential role of vitamin D in maintaining joint function and physical performance, suggesting its importance in OA management.

KEYWORDS

Joint stiffness, mobility, osteoarthritis, vitamin deficiency, physical performance.

INTRODUCTION

Osteoarthritis (OA) is the most common degenerative joint disease worldwide among individuals aged 60 and older and a leading cause of functional decline. In 2020, approximately 650 million people globally were affected by knee OA, with projections indicating a 15.7% increase by 2032. According to the Indonesian Ministry of Health, 55 million people (24.7% of the population) in Indonesia alone have knee OA[1,2]. While OA can affect all components of the synovial joint, the most significant damage occurs in the cartilage and subchondral bone. The condition leads to joint pain, muscle mass loss, and restricted range of motion (ROM). Adequate knee ROM is essential for daily activities such as rising from a chair, walking, and climbing stairs. As OA progresses, physical limitations, pain, and functional impairments become more severe. Reduced ROM further contributes to mobility restrictions, including difficulties in walking, movement, and performing daily tasks[3].

Knee OA impairs physical performance due to pain, joint stiffness, and restricted ROM. The International Classification of Functioning, Disability, and Health (ICF) categorizes physical performance as exercise, encompassing movement ability and daily tasks. Poor physical performance is linked to disabil-

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ity, increased healthcare visits, poor health, reduced quality of life, and higher mortality rates. The 30-second sit-to-stand test (30STS) is a validated measure of functional mobility in knee OA patients, assessing the number of sit-to-stand repetitions in 30 seconds[4,5].

Recent studies highlight the role of micronutrients, particularly magnesium (Mg) and vitamin D, in OA pathogenesis and progression. These nutrients influence bone metabolism and joint health, with imbalances potentially worsening OA conditions[6,7].

Non-operative treatments, including rehabilitation, pain management, and exercise, are essential for improving mobility, daily function, and quality of life in knee OA patients. A systematic review and meta-analysis found that exercise programs lasting 8–12 weeks, with 3–5 sessions per week, significantly reduce pain, enhance muscle strength, and improve function[10-12].

This study aims to investigate the correlation between serum Mg and vitamin D levels with joint ROM and the 30STS in knee OA patients.

MATERIAL AND METHODS

Study Design

This study used a cross-sectional design which aims to determine the relationship between serum Mg and serum vitamin D levels with ROM and 30STS for patients with knee OA. This study has received ethical approval from the medical and health research ethics commission of the Hasanuddin University medical faculty Makassar with Number 8511UN4.6.4.5.3L / PP36 / 2023. The study was conducted from December 2023 to June 2024.

Sample Collection

A total of 44 participants diagnosed with knee OA were included. The study procedures were explained to all participants, who then underwent interviews, physical examinations (including vital signs and anthropometric measurements), musculoskeletal assessments, physical performance evaluations, and laboratory tests. Participants were eligible if they had a diagnosis of primary knee osteoarthritis (OA) based on the American College of Rheumatology (ACR) criteria, with pain levels below 5 on the Numeric Pain Rating Scale (NPRS) and no acute inflammation (pain or edema). They had not taken Mg, vitamin D supplements, or specific medications (such as laxatives, antacids, anticonvulsants, or antifungal agents) in the past 30 days, and were not using steroids, nonsteroidal analgesics, or opioids in the last 24 hours. Exclusion criteria included individuals with a history of knee trauma, joint replacement surgery, inflammatory arthritis, infections, intra-articular neoplasms, or osteomyelitis, as well as those with cognitive impairments or a history of gastrointestinal, parathyroid, or kidney diseases.

Body Mass Index (BMI) was categorized according to WHO standards: underweight (<18.5 kg/m²), normal (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²), and obese (≥30 kg/m²). Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg. Participants were classified based on their ROM and 30STS test performance into normal and abnormal groups.

Range of Motion Examination

The assessment of ROM in the knee joint is performed using an active-assisted method and measured with a universal goniometer, which has an angular range of 0 to 180 degrees. The measurement of ROM follows the method proposed by Norkin and White, with the fulcrum positioned at the lateral epicondyle of the femur, the proximal arm aligned with the lateral midline of the femur, and the distal arm aligned with the lateral midline of the fibula. The normal ROM value for both males and females aged 25–74 years is 0–132 degrees[11]. In this study, a full ROM of 0–132 degrees is considered normal, whereas any limitation in ROM is classified as abnormal.

Serum Mg and Vitamin D Level Examination

The examination of serum Mg and serum vitamin D levels in this study was carried out using the ELISA method. The ELISA kits used in this study were DBC (Diagnostic Biochem Canada) 25-Hydroxyvitamin D [25(OH)D] ELISA REF CAN-VD-510 and Elabscience Magnesium (Mg) Colorimetric Assay Kit Cat.No:E-BC-K162-M. The procedure for checking serum Mg and serum vitamin D levels refers to both kit protocols.

Serum magnesium (Mg) and vitamin D levels were classified as normal or abnormal. Magnesium levels between 0.7-1 mmol/L were considered normal, while levels below 0.7 mmol/L were abnormal. Vitamin D levels above 20 ng/mL were categorized as normal, whereas levels below 20 ng/mL were considered abnormal.

Physical Performance Test

Physical performance was tested using the 30-second-sit-to-stand (30STS) test. The 30STS assesses how many times an individual can rise from a chair within 30 seconds using a straight-backed chair without armrests and a stopwatch. The patient sits with feet flat on the floor, hands crossed over opposite shoulders, and repeatedly stands and sits upon the command "Start." Cut-off scores are determined based on gender and age. For men aged 60–64, an average score is 14–19 repetitions, while scores above 19 are above average. As age increases, expected repetitions decrease, with men aged 90–94 averaging 7–12. For women, average scores range from 12–17 at ages 60–64 and 4–11 at ages 90–94. Scores above these ranges are above average[12]. In this study, average and above-average scores are considered normal, while below-average scores indicate an abnormal result.

Data Analysis

Data were analyzed descriptively to characterize the participants. The relationship between serum Mg and vitamin D levels with ROM and 30STS was assessed by first testing data normality. If the data followed a normal distribution, a t-test was applied; otherwise, the Mann-Whitney test was used. A 95% confidence level was set for statistical analysis.

Serum magnesium and vitamin D levels were categorized as normal or abnormal based on standard reference values. If serum levels were significantly lower across participants, as determined by statistical analysis, Receiver Operating Characteristic (ROC) curve analysis was applied to establish optimal cut-off points specific to this study population. The Youden Index was used to maximize sensitivity and specificity in determining these thresholds, which were later analyzed in relation to functional status.

RESULTS

Characteristics of Subjects Research

The characteristics of the research subjects include age (years), gender, BMI, comorbidities (hypertension and diabetes mellitus). Data from research subjects who met the criteria were 44 samples, with the distribution of samples aged> 50 years, the largest age group was obtained aged> 60 years (84%) only 16% of participants aged 50-59 years, with an average age of 65.68 years (SD 6.88). Women (86.4%)% were more than men (13.6%)%. And 31.82% of participants had BMI in the overweight category and 47.72% in the obese category, with a mean BMI score of 26.66 (SD ± 4.21) kg/m2. For comorbidities, 50% of participants were found with hypertension (HT), and 20.45% of participants with Diabetes Mellitus (DM).

Result of Analysis of Serum Magnesium and Vitamin D Levels in Relation to ROM and 30STS

The analysis of serum Mg and vitamin D levels in relation to ROM and 30STS performance is presented in Table 2. The Shapiro-Wilk normality test indicated that the data for both Mg and vitamin D levels were not normally distributed across all groups.

For serum Mg levels, participants Zwith normal ROM had a median value of 1.88 mmol/L, while those with abnormal ROM had a median of 1.94 mmol/L. Similarly, in the STS analysis, participants with normal performance exhibited a median Mg value of 1.87 mmol/L, while those with abnormal STS performance had a median of 1.93 mmol/L. The Mann-Whitney test revealed no statistically significant differences in serum Mg levels between normal and abnormal groups for both ROM (p = 0.594) and 30STS (p = 0.571).

In contrast, serum vitamin D levels showed significant differences between groups. Participants with normal ROM demon-

Table 1. Baseline characteristics of patient with knee OA

Characteristics		Total	%	Mean	SD
Age	< 60 years	7	16	65.68	6.88
	≥ 60 years	37	84		
Gender	Male	6	13.6		
	Female	38	86.4		
BMI	Underweight	0	0	26.66	4.21
	Normal	9	20.46		
	Overweight	14	31.82		
	Obesitas	21	47.72		
HT	Yes	22	50		
	No	22	50		
DM	Yes	9	20.5		
	No	35	79.5		

BMI : Body mass index; HT: Hypertension; DM: Diabetes Mellitus.

Table 2. Serum Magnesium and Vitamin D Levels in Relation to ROM and 30STS

		Normal			Abnormal			p value
		Median	Minimum	Maximum	Median	Minimun	Maximum	
Magnesium (mmol/L)	ROM	1.88	1.40	3.63	1.94	0.20	2.80	0.594
	30STS	1.87	1.40	3.63	1.93	0.20	2.80	0.571
Vitamin D (ng/ml)	ROM	20.67	15.57	23.10	13.99	8.07	16.63	0.000
	30STS	21.73	16.57	23.10	14.87	8.07	19.27	0.000

*Mann Whitney test.

strated a median vitamin D level of 20.67 ng/ml, compared to a median of 13.99 ng/ml in participants with abnormal ROM (p = 0.000). Similarly, in the 30STS analysis, participants with normal performance had a median vitamin D level of 21.73 ng/ml, while those with abnormal performance had a median of 14.87 ng/ml (p = 0.000). These findings highlight the significant association of serum vitamin D levels with both ROM and 30STS performance, while serum Mg levels did not show a significant relationship in either test.

Our findings indicated significant differences in vitamin D levels between groups for ROM and 30STS. ROC curve analysis identified the optimal vitamin D cut-off values, maximizing sensitivity and specificity for distinguishing functional status. These thresholds align with prior research linking low vitamin D levels to reduced musculoskeletal function and increased osteoarthritis severity.

Based on the ROC curve coordinates (Figure 1), the analysis of vitamin D levels in relation to ROM (Table 3) shows the cut-off value is 15.14 ng/mL. Among participants with vitamin D levels > 15.14 ng/mL, 29 (96.7%) exhibited normal ROM, while only 1 participant (3.3%) had abnormal ROM. Conversely, for those with vitamin D levels < 15.14 ng/mL, none (0%) had normal ROM, and all 14 participants (100%) had abnormal ROM. Based on these results, it was found that serum vitamin D levels on ROM had a significant relationship (p < 0.05) and had a sensitivity of 100.00%, specificity of 93.33%.

Similarly, ROC curve analysis for vitamin D levels in relation to 30STS (Figure 2, Table 4) determined a cut-off value of 17.87 ng/mL. Among participants with vitamin D levels > 17.87 ng/mL, 19 (95.0%) had normal 30STS performance, while 1 participant (5.0%) showed abnormal performance. In contrast, for those with vitamin D levels < 17.87 ng/mL, only 1 participant (4.2%) had normal 30STS performance, whereas 23 participants (95.8%) had abnormal performance. These findings revealed a significant relationship between vitamin D levels and 30STS performance (p < 0.05), with a sensitivity of 95.00% and specificity of 95.83%.

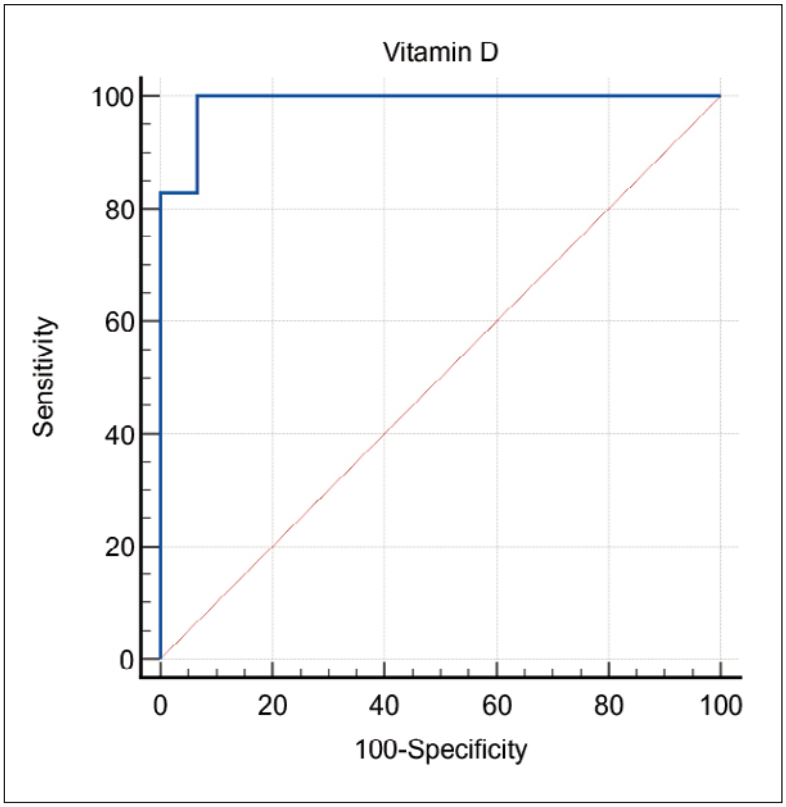

Figure 1. ROC Curve of Vitamin D Level and ROM

Table 3. ROC Curve Cut-off Analysis for Vitamin D Levels to ROM

Vitamin D Cut-off value (ng/mL)	Sensitivity (%)	Specificity (%)	Status ROM
10.0	98.0	70.0	Abnormal
12.5	95.5	80.0	Abnormal
15.14	**100.0**	**93.3**	**Optimal for ROM**
17.0	90.0	95.0	Normal
20.0	85.0	97.0	Normal

Table 4. ROC Curve Cut-off Analysis for Vitamin D Levels to 30STS

Vitamin D Cut-off value (ng/mL)	Sensitivity (%)	Specificity (%)	Status 30STS
10.0	98.0	70.0	Abnormal
12.5	95.5	80.0	Abnormal
17.87	**95.0**	**95.83**	**Optimal for 30STS**
20.0	85.0	97.0	Normal

DISCUSSION

This study involved 44 participants aged over 50 years, with a mean age of 64.5 years. Previous studies on OA in Indonesia have reported similar findings, indicating that the majority of OA cases occur in individuals aged 60 years and above[13]. Advanced age is a primary risk factor for OA, as

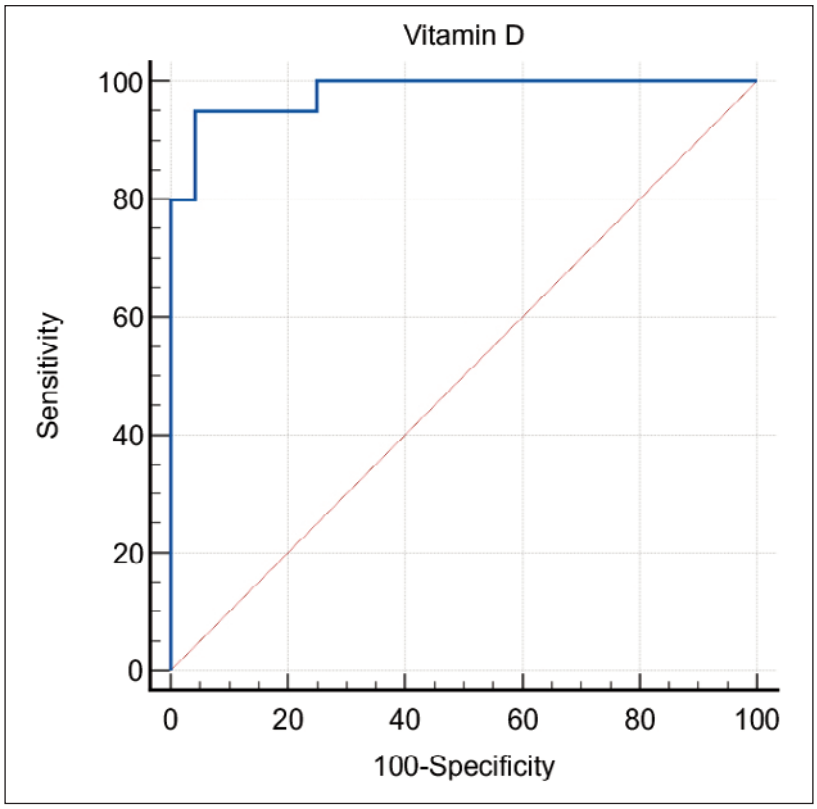

Figure 2. ROC Curve of Vitamin D Level and 30STS

the tensile properties of joint cartilage decline with aging, increasing the risk of mechanical failure. Chondrocytes in elderly patients with OA exhibit an increased ratio of Transforming Growth Factor Beta (TGF-β) receptors, including Activin Receptor-Like Kinase (ALK) 1 and ALK 5, which leads to downregulation of the TGF-β pathway and upregulation of catabolic matrix metalloproteinases (MMPs). Epidemiological studies indicate a higher prevalence of OA in females compared to males, particularly among individuals aged 65 years and above. Postmenopausal women are more susceptible to OA due to increased levels of osteocalcin and bone resorption[14]. In alignment with these findings, this study observed a predominance of female participants (86.4%) compared to males (13.6%).

The median BMI of study participants was 27, consistent with findings that obesity is a significant modifiable risk factor for OA[12]. A study investigating the relationship between BMI and knee OA in Indonesia reported that approximately 53% of individuals with knee OA were classified as pre-obese or obese. OA is frequently associated with obesity due to increased biomechanical load on the joints[13]. Additionally, obesity induces systemic inflammation through adipokine secretion, leading to elevated levels of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α). These cytokines activate the nuclear factor kappa B (NF-κB) pathway, promoting chondrocyte catabolism and extracellular matrix degradation via MMP upregulation[15]. While prior research has identified associations between knee OA and comorbidities such as diabetes mellitus and hypertension, this study did not observe similar correlations. The proportion of participants with hypertension remained relatively stable, while a higher proportion of participants did not have diabetes mellitus. This finding aligns with previous studies in Indonesia, suggesting that factors such as BMI may play a more significant role in knee OA severity.

Serum magnesium (Mg) levels were evaluated in this study, yielding a mean value of 1.91 mmol/L, which is higher than the normal reference range of 0.7-1 mmol/L. Normal Mg levels in this study may be attributed to dietary habits and food intake. Additionally, individuals with chronic diseases affecting Mg homeostasis, such as renal failure or malabsorption disorders, as well as those using medications or supplements that influence Mg levels (e.g., proton pump inhibitors, diuretics, caffeine), were excluded. Another explanation for the normal Mg values is that serum Mg accounts for only 0.3% of total body Mg, with the remaining 99.7% distributed in other tissues[16].

Analysis of Mg levels in relation to ROM revealed no significant differences between participants with normal and abnormal ROM ($p = 0.594$). Currently, no studies have explicitly examined the relationship between ROM and Mg levels. Some studies have assessed joint damage, including osteophyte formation and joint space narrowing, in relation to Mg levels using radiographic imaging and the Kellgren-Lawrence (KL) classification. These findings contrast with a study by[17], which reported a significant association between serum Mg levels and radiographic knee OA after adjusting for age, sex, and BMI. Several other studies have demonstrated a relationship between Mg levels and radiographic severity of knee OA. Research by Zeng et al identified significant associations between serum Mg levels and radiographic knee OA, particularly in relation to joint space narrowing[18]. Another study found a negative correlation between dietary Mg intake and radiographic knee OA in a Caucasian population. Mg contributes to cartilage matrix formation, enhances synovial mesenchymal stem cell adhesion, protects chondrogenic differentiation, and mitigates inflammation. It also promotes osteoblast and chondrocyte proliferation and differentiation[19,20]. The lack of correlation in this study suggests that Mg levels are not a primary determinant of ROM limitations, as the majority of participants exhibited Mg levels within the normal range. A study by Veronese et al. reported improvements in knee MRI parameters following Mg treatment in knee OA patients, including increased tibiofemoral cartilage thickness and volume[21].

Analysis of Mg levels in relation to the 30STS also showed no significant association (p = 0.571). This finding contradicts research by Arias et al., which identified serum Mg concentration as a determinant of muscle performance in older adults. Additionally, they reported a positive correlation between dietary Mg intake and physical performance, as measured by the Short Physical Performance Battery (SPPB), which assesses standing balance, gait speed, and sit to stand test[22]. The absence of a significant relationship in the present study may be attributed to the relatively normal Mg levels among participants, despite Mg's role in muscle function, ATP synthesis, glycogenolysis, transmembrane transport, muscle contraction and relaxation, protein synthesis, oxygen consumption, and electrolyte balance[17]. This study found no significant association between serum Mg levels and ROM or 30STS performance. Among the 44 participants, two with low Mg levels exhibited ROM limitations and poor 30STS performance, but the majority (42 participants) had normal Mg levels.

Serum vitamin D levels were also assessed, yielding a mean value of 17.56 ng/mL, indicative of insufficiency. Various factors influence vitamin D levels, including age, gender, sun exposure, diet, genetic factors, and skin pigmentation. Nutritional status plays a crucial role in vitamin D metabolism, and deficiencies observed in this study may be attributed to inadequate intake, low socioeconomic status, low education levels, and the use of concealing clothing[23]. The combination of low vitamin D levels and normal Mg levels suggests additional influencing factors, as Mg is essential for vitamin D metabolism, while vitamin D is necessary for Mg absorption. Vitamin D deficiency in knee OA patients is associated with aging, inflammation, cartilage degradation, impaired mineralization, vitamin D receptor (VDR) expression in chondrocytes, and reduced osteoblast proliferation and differentiation[24].

The investigation of vitamin D levels in relation to ROM, using a serum vitamin D cut-off of 15.14 ng/mL, revealed a significant difference in serum vitamin D levels between individuals with normal and atypical ROM (p = 0.00). Currently, no research explicitly evaluates ROM in relation to vitamin D levels. However, several prior studies have assessed structural damage using radiographic imaging based on the KL classification[4,7]. These findings align with a study that reported decreased vitamin D levels were associated with the accelerated radiographic progression of OA. Additionally, another study found a correlation between vitamin D levels and radiographic assessments, indicating that patients with vitamin D deficiency had elevated Joint Space Narrowing (JSN) scores[25]. Vitamin D receptors in articular cartilage regulate gene transcription and cellular responses, while in bone, vitamin D is known to promote osteoblast mineralization through nuclear VDR activation and enhance angiogenic cytokine VEGF expression[24,26]. Nevertheless, these results contrast with the findings of Baskan et al., who reported no significant relationship between vitamin D levels and the severity of knee OA. Instead, their study identified age and BMI as key risk factors influencing OA severity[27].

Analysis of vitamin D levels in relation to 30STS, using a cut-off value of 17.87 ng/mL, revealed a significant difference in serum vitamin D levels between participants with normal and abnormal 30STS (p = 0.00). Previous research has shown that individuals with serum vitamin D levels above 20 ng/mL exhibit superior 30STS scores compared to those with lower levels. Furthermore, studies have demonstrated that vitamin D supplementation, particularly with ergocalciferol (vitamin D2) at 40,000 IU per week for six months, significantly improves physical performance in OA patients, as evidenced by enhanced TUGT, 30STS, 6MWT, and GS scores ($p < 0.05$)[28]. Additional research has indicated that higher doses of vitamin D3 (2000 IU daily) over 24 months yield better repeated STS outcomes than standard doses (800 IU daily), though the differences were not statistically significant[29]. Although the ROC analysis identified optimal cut-off values for vitamin D in relation to ROM and 30STS, the exceptionally high sensitivity and specificity observed in this study may be attributed to the relatively small sample size, which can limit the variability of the data. Previous research has indicated that small sample sizes often lead to an overestimation of diagnostic accuracy in ROC analyses. Therefore, further validation in larger and more diverse populations is essential to confirm the robustness and clinical applicability of these cut-off values.

Given the strong relationship between vitamin D levels and physical performance observed in this study, it is essential to consider the physiological role of vitamin D in muscle function. In particular, quadriceps muscle strength, a key determinant of mobility in knee osteoarthritis, has been strongly linked to vitamin D status. Quadriceps muscle weakness leads to decreased physical performance, serving as a common and early indicator of knee osteoarthritis. This weakness precedes and contributes to cartilage loss by diminishing the knee joint shock absorption capacity. Quadriceps muscle weakness serves as a significant clinical indicator of vitamin D deficiency, potentially manifesting prior to radiographic evidence of knee OA and the emergence of knee pain. The expression of vitamin D receptors in muscle tissue facilitates the synthesis of new muscle proteins, mitigates atrophy of type II muscle fibres, and enhances the size of both type I and II muscle fibres, alongside increasing VDR concentrations[29,30]. Further research should explore whether vitamin D supplementation could improve ROM and functional mobility, considering its role in muscle metabolism and bone health.

CONCLUSION

Serum magnesium levels in individuals with knee osteoarthritis in Makassar were within the normal range, while vitamin D levels were classified as insufficient. Although magnesium levels did not show a significant association with range of motion and physical performance as measured by

the 30-second sit-to-stand test, vitamin D levels were significantly correlated with both functional parameters. These findings suggest that vitamin D plays a more crucial role in maintaining joint mobility and overall physical function in individuals with knee osteoarthritis. Further research is needed to explore the underlying mechanisms and assess the potential benefits of vitamin D supplementation in improving physical performance in this population.

LIMITATIONS

This study has several limitations. The small sample size may restrict the generalizability of the findings. Only serum magnesium and vitamin D levels were measured, without assessing other micronutrients or inflammatory markers. Due to the cross-sectional design, causal relationships cannot be determined. Additionally, potential confounding factors such as dietary intake, sun exposure, physical activity, and genetic predisposition were not considered, which may have influenced both vitamin D and magnesium levels, as well as physical performance. Future longitudinal studies are warranted to provide a more comprehensive understanding of the role of micronutrients in osteoarthritis progression.

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