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Serum Vitamin D Level in Patients with Knee Osteoarthritis: Relationship to Clinical and Radiological Severity

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Abstract

Background: Osteoarthritis (OA) is a common joint condition that typically causes chronic pain. OA has been associated with degenerative changes. Vitamin D deficiency might be associated with OA and therefore warrants routine screening of such patients.

Aim of Study: To identify serum level of vitamin D in patient with primary Knee OA (KOA) in comparison with normal control group and to evaluate the association between the level of vitamin D and the clinical severity and the radiological severity.

Material and Methods: Case control study, carried on 90 individuals, 45 cases (patients having knee OA) and 45 healthy control, and vitamin D level was assessed by ELIZA in both groups.

Results: Cases with OA had statistically significant lower levels of vitamin D compared to control group. The mean serum vitamin D among cases is 20.94±9.23ng/ml versus 32.72±12.56 among control group. Vitamin D had moderate discriminating ability for differentiation between OA cases and healthy group.

Conclusion: There was significant negative correlation between vitamin D level and clinical and radiological severities of KOA patients.

Key Words: *Vitamin D – OA – ELIZA.*

Introduction

OSTEOARTHRITIS (OA) is a chronic disease that affect the joint leading to progressive damage to articular cartilage and, subsequently, to the subchondral bone and surrounding structures. OA is a disabling condition with increasing incidence and prevalence in the general population **[1]**. Different joints can be involved, yet most social OA burdens are related to hip and knee OA. OA risk increases with age and is greater among women compared with men. There is a clear association of overweight with increased risk for OA, particularly at the knee; one systematic review found that obesity increased the risk of OA about 3-fold **[2]**. The role of specific vitamins and diet also continues to be an active research area. Vitamin D has been the most extensively studied **[3]**. KOA primarily manifests as knee joint pain that is aggravated by increase loading, and relieved after rest or the application of heat. Patients typically experience either dull and needle-like pain or joint soreness **[4]**. Diagnostic criteria were developed for OA of the knee. Lequesne has proposed sets of clinical criteria for OA in several specific joints **[5]**. The diagnosis of osteoarthritis (OA) has most often been based on radiographic appearance, rather than clinical features. Radiographic criteria were proposed by Kellgren and Lawrence in 1957, and those criteria were later accepted by the World Health Organization at a symposium held in Milan in 1961. The primary goal of pharmacological OA therapy is to alleviate the symptoms of OA. Nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics are the most frequently prescribed medications in this category. Topical and intra-articular therapies have also been used. Palliative pain management is the primary focus of current OA management strategies. Surgical management is also alternative non pharmacological treatment among eligible patients **[6]**. Joint replacement surgery has been shown to relieve the disease's excruciating and disabling symptoms in acute circumstances. Currently, no treatments are available to slow or reverse the development of OA

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[7]. Vitamin D is fat soluble vitamin, exists in two major forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) [8]. The Endocrine Society, the National and International Osteoporosis Foundation, and the American Geriatric Society define vitamin D deficiency as the level of 25-hydroxyvitamin (25 OH D) of less than 30ng/mL. Vitamin D deficiency levels are: Severe deficiency: <12ng/mL = <30nmol/L, Deficiency: <20ng/mL = <50nmol/L, Insufficient: 20–29 ng/mL = 50–75 nmol/L, Normal: 30–50ng/mL = 75–125nmol/L [9]. The majority of patients with vitamin D deficiency are asymptomatic. However, even mild chronic vitamin D deficiency can lead to chronic hypocalcemia and hyperparathyroidism, which can contribute risk of osteoporosis, falls, and fractures, especially in older individuals [10]. Vitamin D exerts both direct and indirect actions on bone. Vitamin D is a major determinant of mineral homeostasis, promoting intestinal calcium and phosphorus absorption, which are required for optimal mineralization of bone. The direct actions of vitamin D on bone are more complex to demonstrate [11]. Receptors of Vitamin D have been demonstrated in the human chondrocytes. Through these receptors, vitamin D may regulate matrix metalloproteinase and prostaglandin E2 production [12]. Vitamin D has been shown to have a direct impact on cartilage via vitamin D receptors by metabolic transformation, which stimulates proteoglycan synthesis in mature chondrocytes [13]. According to recent studies, vitamin D deficiency is directly linked to oxidative stress damage and mitochondrial abnormalities, which worsens osteoarthritis (OA) disease and increases pain [14].

Patients and Methods

This study was case control study, carried on 90 individuals, 45 cases (patients having knee OA) and 45 healthy control (matched for age and sex with cases). From the period Octoper / 2023 to Octoper / 2024.

It was carried out at Mansoura University after obtaining the approval of the institutional research board (IRB) Committee of Faculty of Medicine, Mansoura University.

Ethical consideration: The Faculty of medicine, Mansoura University Medical Ethics Research Committee reviewed and authorized the study protocol. All the participants were given a full explanation of the study and were informed about the consent form and assigned it. Inclusion criteria: Patients with knee osteoarthritis diagnosed based on the clinical and radiological criteria of American Collague of Rheumatology (ACR) [15]. Exclusion criteria: Secondary OA due to deformity, trauma, Metabolic disorder such as DM, thyroid disorder, Renal failure with or without dialysis (affect vitamin D activation), Patient on medication for osteoporosis except for calcium supplementation and Extreme obesity (BMI >35).

Methods: All patients subjected to the following: Personal history, Complaint and its duration. Onset, course, duration of complaint. A detailed history of present medications past history of Serious diseases or previous medications.

Clinical examination: Inspection: While standing, for presence of swelling, Popliteal fossa for Baker's cysts, Erythema, Muscle atrophy, Deformities, Skin changes and scars. While supine: Localized swelling may indicate bursitis, tendon, or ligament pathology.

Palpation: With knee extended: Temperature, Patella, Popliteal fossa, swelling and Bursa detection. With knee flexed to 90°: Femoral condyles, Medial and lateral joint line and Medial collateral ligament and Palpation for Effusion.

Range of motion (ROM) Power assessment: For knee muscle flexors and extensors.

Clinical severity score: (WOMAC) Western Ontario and McMasters Universities Index of Osteoarthritis (WOMAC) [16]. In WOMAC Patients were asked to answer each question with regard to the pain, stiffness, or difficulty experienced in the previous 48 hours. Visual analogue scale (VAS): 10cm VAS with the 0cm point suggesting no pain and the 10cm point indicating the worst possible pain. Lequesne Algofunctional Index [17]. The Lequesne Algofunctional Index of Knee Osteoarthritis (LAIKOA) was initially referred to as the Index for the severity of knee OA and it was developed originally in French language in the 1970s. It consists of 11 items in three domains which are pain or discomfort (5 items), maximum walking distance (2 items), and ADL or function (4 items). A modification of a 1991 version was to have the duration of morning stiffness scored 0 if it was 1 minute or less and 1 if it was from 1 to less than 15 minutes. Pain on walking in a 1991 version expanded "early after starting" to "after initial ambulation and increasingly with continued ambulation". Index of severity = SUM (points for all parameters). Interpretation: Point of each domain range from (0 - 8). The sum for score range from (0 - 24).

Radiological severity score: Kellgran-Lawrence (KL) (Kellgren, et al., 2013): It was evaluated by anteroposterior knee X-ray examination with

patient in standing position and was graded according to scale (Grade 1-4).

Assessment of serum vitamin D level by ELISA: The kit uses a double antibody sandwich enzyme-linked immunosorbent assay (ELIZA) to assay the level of human vitamin D3 in samples. Catalogue No. (201-12-1547). Add vitamin D3 to monoclonal antibody, incubation: Then, add vitamin D3 antibodies labelled with biotin, and combined with streptavidin-HRP to form immune comples: Then carry out incubation and washing again to remove the uncombined enzyme. Add chromogen solution A, B, to the color of the liquid changes into blue, and at the effect of the acid, the color become finally yellow. The Chroma of color and the concentration of the human substance vitamin D3 of sample were positively correlated.

Sample size calculation: It was based on difference frequency of vitamin D deficiency between cases with osteoarthrtis and control group retrieved from previous research **[18]**. Using G*power version 3.0.10 to calculate sample size based on difference of 53.3, 2-tailed test, α error = 0.05 and power 90.0% then total sample size will be 16 samples in each group at least.

Results

Mean age of the studied cases is 43.87±6.88 years for cases versus 43.20±7.38 years for control group without statistically significant difference between them (p=0.659). Table (1).

Table (1): The demographic characteristics of the studied groups.

	Cases N=45	Control N=45	Test of significance
Age/years:			
Mean ± SD	43.87±6.88	43.20±7.38	t=0.443
			p=0.659
Sex n (%):			
Male	3 (6.7)	0	FET=3.10
Female	42 (93.3)	45 (100)	p=0.242
Disease duration (years):			
Mean ± SD	10.31±2.73	NA	
Median (min-max)	10 (6-18)		
BMI (Kg/m^2):			
Mean ± SD	38.28±1.13	37.40±2.99	t=1.84
			p=0.07

There was significant higher mean serum vitamin D among control than cases. Mean serum vitamin D among cases is 20.94±9.23ng/ml versus 32.72±12.56 among control group (p=0.001). Fig. (1).

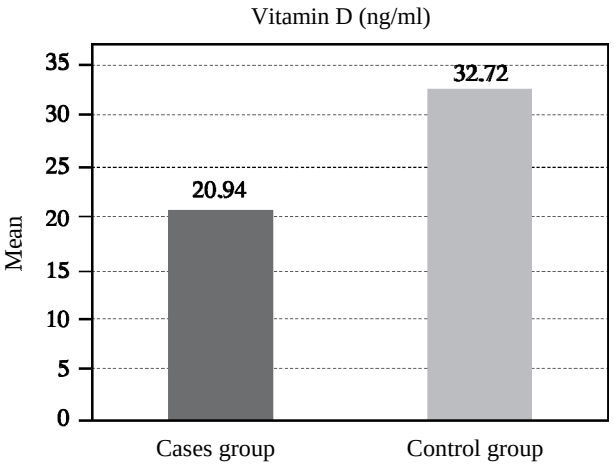

Fig. (1): Mean serum vitamin D among studied groups.

Mean WOMAC score is 69.07±8.44 ranging from 48 to 73. Mean WOMAC A score is 13.98±3.96 ranging from 5 to 16, mean WOMAC B score is 6.53±1.09 ranging from 4 to 7 and mean WOMAC C score is 47.02±4.85 ranging from 37 to 50 and mean Lequesne index is 14.84±2.82 ranging from 9 to 17. Median VAS score is 7 ranging from 5 to 9. Table (2).

Table (2): The disease characteristics among studied cases.

	Cases N=45 Mean ± SD Median (min-max)
WOMAC (96)	69.07±8.44 73 (48-73)
WOMAC A (0 - 20)	13.98±3.96 16 (5-16)
WOMAC B (0 - 8)	6.53±1.09 7 (4-7)
WOMAC C (0 - 68)	47.02±4.85 50 (37-50)
Lequesne index	14.84±2.82 17 (9-17)
VAS score median (min-max)	7.0 (5.0-9.0)

Tenderness scoring system distribution among studied cases is as the following: 77.8% grade 2, 17.8% grade 1 and 4.4% grade 3. Radiological severity grade is as following: 11.1% grade 1,75.6% grade 2 and 13.3% grade 3. Figs. (2,3).

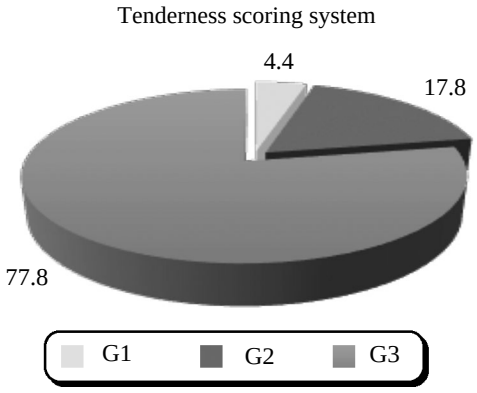

Fig. (2): Pie chart showing tenderness scoring system.

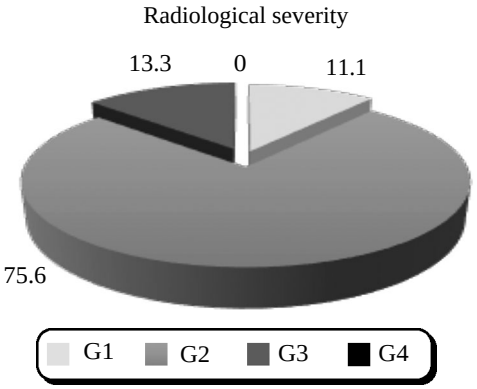

Fig. (3): Pie chart showing radiological severity.

The area under ROC curve for serum vitamin D in differentiating cases from control group is good (AUC=0.768) with the best detected cut off point is 21.6ng/ml yielding sensitivity 62.2%, specificity 86.7% and total accuracy 74.4%. Table (3), Fig. (4).

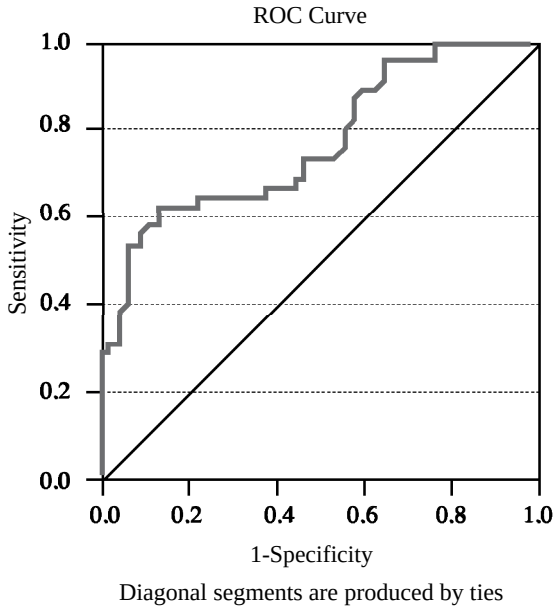

Fig. (4): ROC curve of serum vitamin D level in differentiating between cases and control groups.

Table (3): The validity of serum vitamin D in differentiating between cases with knee osteoarthritis and control group.

	AUC (95%CI)	*p*-value	Cut off point	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Vitamin D	0.768 (.671-0.86)	0.001*	≤21.60ng/ml	62.2	86.7	82.4	69.6	74.4

There was statistically significant negative correlation between serum vitamin D level and VAS score (r=–0.830), radiological severity (r=–0.377), TTS (r=–0.769), WOMAC score (r=–0.693). Figs. (5-9).

There was statistically significant higher mean serum vitamin D among cases with grade 1 tenderness scoring system than grade 2 and grade 3 (34.41±2.05, 18.86±6.65 and 3.50±0.001. A statistically significant higher mean serum vitamin D among cases with radiological severity score grade 1 followed by grade 2 and grade 3 (27.46±10.43, 21.27±8.07 and 13.62±11.17, respectively. Table (4), Figs. (10,11).

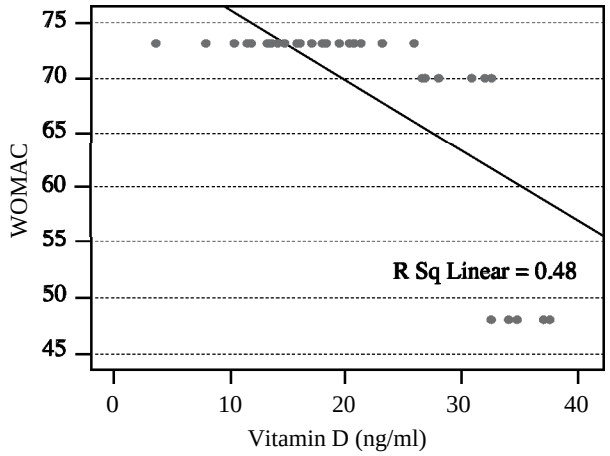

Fig. (5): Scatter diagram showing correlation between serum vitamin D and WOMAC score among studied cases.

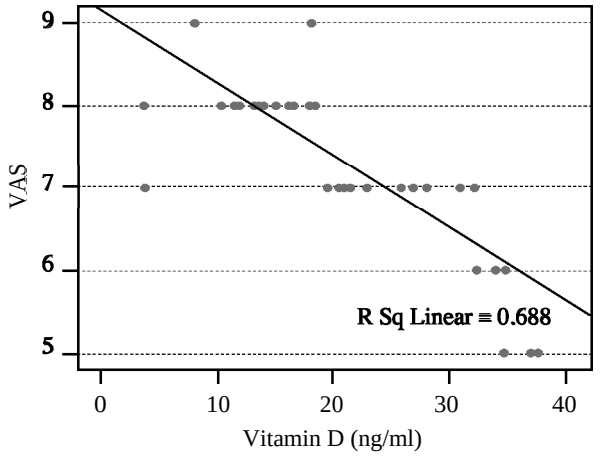

Fig. (8): Scatter diagram showing correlation between serum vitamin D and VAS score among studied cases.

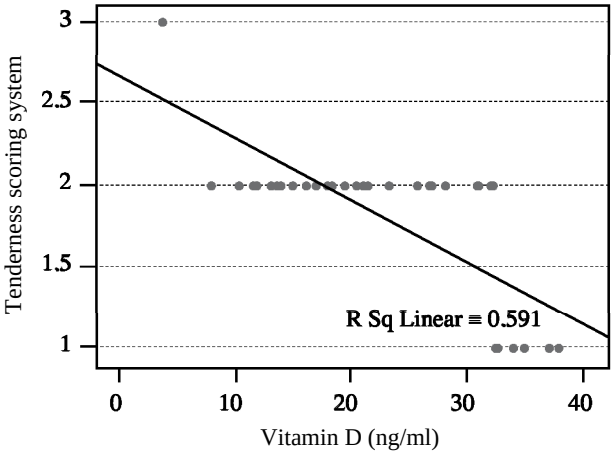

Fig. (6): Scatter diagram showing correlation between serum vitamin D and tenderness scoring system among studied cases.

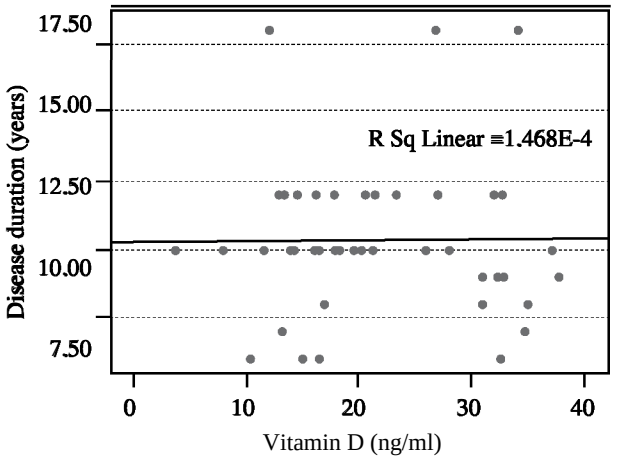

Fig. (9): Scatter diagram showing correlation between serum vitamin D and disease duration among studied cases.

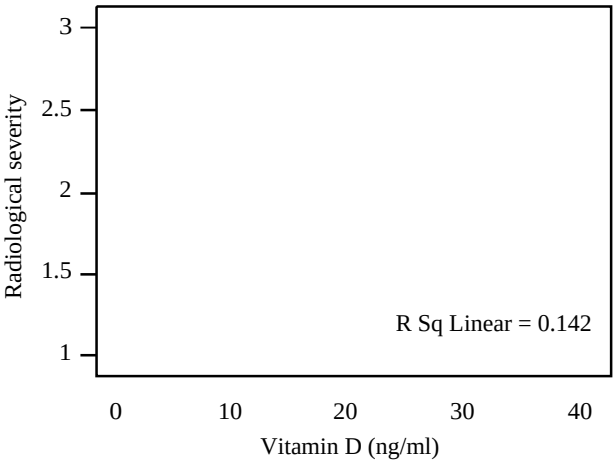

Fig. (7): Scatter diagram showing correlation between serum vitamin D and radiological severity grade among studied cases.

Table (4): The relation between serum vitamin D and clinical and radiological disease severity score.

	Vitamin D Mean ± SD	*p*-value
TTS:		
G1	34.41±2.05	<0.001*
G2	18.86±6.65	
G3	3.50±0.001	
Radiological severity:		
G1	27.46±10.43	0.038*
G2	21.27±8.07	
G3	13.62±11.17	

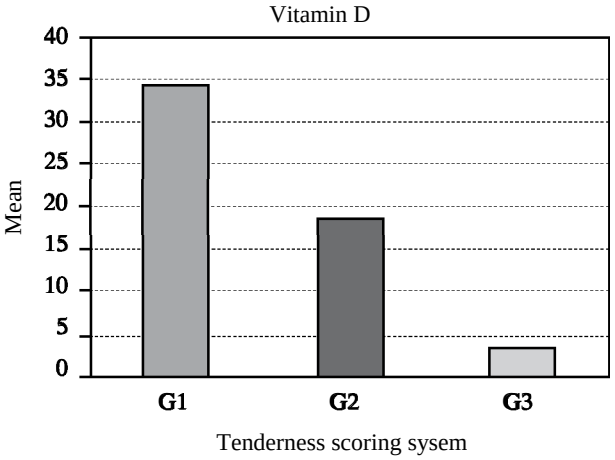

Fig. (10): Serum vitamin D distribution according to tenderness scoring system among studied cases.

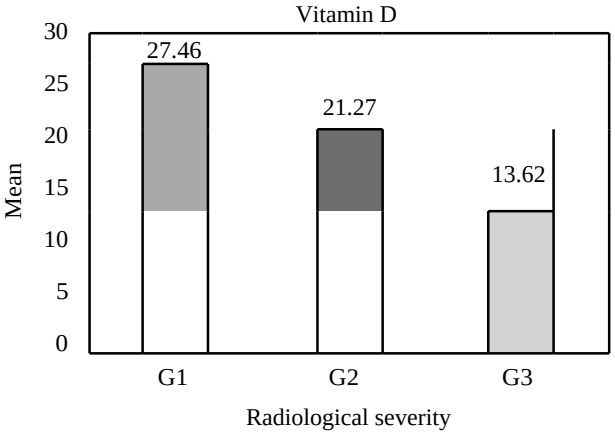

Fig. (11): Serum vitamin D distribution according to radiological severity grades among studied cases.

Discussion

Osteoarthritis (OA) is the most common form of arthritis in the world, it is a degenerative joint disease of the knee. It can be classified into 2 categories: Primary osteoarthritis and secondary osteoarthritis **[19]**. The current study revealed that cases with OA had lower levels of vitamin D compared to control group which was statistically significant. The mean serum vitamin D among cases is 20.94±9.23 ng/ml versus 32.72±12.56 among control group. The exact role of vitamin D in bone metabolism and on chondrocytes has been previously studied. Suboptimal levels of vitamin D have adverse effects on calcium metabolism, osteoblastic activity, matrix ossification, and bone mineral density. A direct effect of vitamin D metabolites on articular chondrocytes, subchondral bone quality, and early degenerative changes could, therefore, increase the susceptibility to OA **[20]**. In previous report by Anari et al., revealed that the average serum vitamin D in OA patients and controls were 26.8±6.2ng/ml and 28.1±5.3ng/ml, respectively (p=0.36). There was a significant association between serum vitamin D and staging of knee OA (p=0.001). Based on vitamin D levels, most of patients with vitamin D deficiency were in stages III and IV **[21].** Which agreed with our results. In same line with Tripathy et al., who found that the average serum vitamin D level in normal healthy individuals was higher than cases with OA knee **[22].** In addition, Afzal et al., had revealed that the rate of vitamin D deficiency was considerably higher in patients with OA knee as compared to controls (70.0% vs. 16.7%) **[18].** Which matches with other report that found the rate of vitamin D deficiency was higher among patients with osteoarthritis of knee joint **[23]**. For vitamin D differentiating cases with OA from control group in the present study, the AUC=0.768 with the best detected cut off point is 21.6 yielding sensitivity 62.2%, specificity 86.7% and total accuracy 74.4%. While Askari et al., logistic regression showed that after adjustment, each unit decrease in vitamin D level leads to an increase the odds ratio of OA to 0.67 and 0.002 times respectively. In receiver operator characteristic (ROC) curve analysis, the cutoff-point for vitamin D was determined 12.68nmol/L. Furthermore, according to the regression models, by decreasing each unit of vitamin D levels, the risk of disease increases about 0.67 times **[24]**. The present study revealed a statistically significant negative correlation between vitamin D level and VAS score, radiological severity, and WOMAC score. There were statistically significant higher mean vitamin D among cases with grade 1 tenderness scoring system than grade 2 and grade 3. A statistically significant higher mean vitamin D among cases with radiological severity score grade 1 followed by grade 2 and grade 3, respectively. Wu et al., found in their meta-analysis that patients with chronic pain, including OA, had a lower average vitamin D concentration than controls. They found that patients with OA had a 20% difference in concentration **[25]**. Patients with a deficiency of vitamin D showed a statistically significant negative strong relationship between vitamin D concentration and pain intensity. The relationship between pain intensity and vitamin D is controversial **[26]**. In addition, Tripathy et al., revealed that the odds of development of KOA were 2.77 times more in younger individuals with inadequate vitamin D compared to healthy individuals with no association of severity of vitamin D deficiency with the clinical and radiological severity of KOA **[22],** which agreed with our results. While Alabajos-Cea et al., revealed about pain intensity levels, the t-test showed statistically significant differences, showing higher levels of pain intensity in patients with lower vitamin D lev-

els. Similarly, statistically significant differences were found in the WOMAC values with a higher score in the deficient group [27]. Also, in previous study, revealed high VDD prevalence was evident in patients who underwent TKA, with 75.0% of the OA group having 25(OH)D levels of <20ng/dL. Moreover, a significant difference was observed between the two groups [28]. In addition, Wang et al., meta-analysis showed that vitamin D could affect the VAS of patients. It was hereby believed that although the VAS of the patients had improved, the reliability of this result should still be further confirmed in subsequent studies. The WOMAC pain scale was more specific and more useful for patients to score their pain levels. The statistical analysis showed that vitamin D supplementation did contribute to the relief of WOMAC pain and the functional improvement in patients with KOA. The improvement in joint function and WOMAC pain reinforced the ability of the vitamin to relieve the pain and improve the life function in patients with KOA [29]. However before concluding, it's worth mentioning that Anari et al., in another similar study reported only insignificantly higher frequency of vitamin D deficiency in Iranian patients with OA knee (27.8% vs. 24.0%) negating any such association. A possible explanation for this conflict among studies can be the overall high prevalence of vitamin D deficiency in Indo-Pakistanian [21].

Limitations of the present study, the existing evidence on association between vitamin D deficiency and knee osteoarthritis contained controversy. The small sample size which based the presenting results. No such published research in local population which compelled the current study, so further multicenter studies should be conducted. The results of the present study revealed association between vitamin D deficiency and knee osteoarthritis, it will warrant routine screening that will enable timely identification of patients with OA knee having vitamin D deficiency allowing better management of such cases in future orthopedic practice.

Conclusion: Serum vitamin D level is significantly low in the patients with KOA patients compared to healthy individuals. There was significant negative correlation between vitamin D level and clinical and radiological severities of KOA patients.

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خشونة المفاصل ودور فيتامين د
فى الوقاية والعلاج

خشونة المفاصل هى حالة مزمنة تُصيب المفاصل وتؤدى إلى تآكل الغضروف المفصلى وتلف تدريجى فى العظم تحت الغضروف، بالإضافة إلى تأثر الأنسجة المحيطة. وتُعد من الأمراض الشائعة التى تُسبب الإعاقة، وتزداد نسبة الإصابة بها مع التقدم فى العمر، كما أنها أكثر شيوعاً بين النساء مقارنةً بالرجال.

تتعدد الأسباب والعوامل التى تُسهم فى تطور خشونة المفاصل، ومن أبرز الاسباب التقدم فى السن والوزن الزائد وخاصة فى حالات خشونة الركبة حيث وجد أن السمنه تُضاعف خطر الاصابة ثلاث اضعاف قلة النشاط البدنى ونمط الحياة غير الصحى تظهر خشونة المفاصل فى صورة ألم متزايد فى المفصل، يزداد مع الحركة أو التحميل، ويخفب الراحة أو استخدام الحرارة. وقد يشعر المريض بألم خفيف أو كالإبر أو إحساس بالتيبُّس أو الطقطقة فى المفصل.

يتم التشخيص بناء على الفحص السريرى والتاريخ المرضى، ويُعتمد فى الغالب على الفحوصات الاشعاعية، مثل المعايير التى وضعها «كيلغرين ولورنس» والتى تم اعتمادها من قبل منظمة الصحة العالمية.

لا توجد حتى الآن أدوية توقف أو تعكس تطور خشونة المفاصل، ويهدف العلاج إلى تخفيف الأعراض وتحسين جودة الحياة. وتشمل الخيارات العلاجية .

- استخدام المسكنات ومضادات الالتهاب.
- العلاجات الموضعية والحقن داخل المفصل.
- العلاج الطبيعى وممارسة التمارين المناسبة.

فى الحالات المتقدمة، يمكن اللجوء إلى التدخل الجراحى مثل استبدال المفصل فيتامين د يُعد من العوامل المهمة فى صحة العظام والمفاصل، حيث يساعد على امتصاص الكالسيوم والفوسفور الضروريين لتقوية العظام. ويوجد فيتامين د فى شكلين رئيسيين: د ٢ود ٣.

نقص فيتامين د قد يؤدى إلى ضعف العظام وزيادة خطر الكسور، ويُعد شائعًا لدى كبار السن. وجدت مستقبلات لفيتامين د فى خلايا الغضروف، مما يدل على تأثيره المباشر على المفاصل من خلال تنظيم إنتاج بعض المواد المؤثرة على صحة الغضاريف، مثل الإنزيمات والبروستاجلاندى ن كما أن نقصه يرتبط بزيادة الإجهاد التأكسدى واضطرابات الميتوكوندريا، مما يؤدى إلى تفاقم الحالة وزيادة الألم.