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The Associations of Vitamin - D Deficiency with Knee Pain and Biomechanical Abnormalities in Young Iranian Patients with Patellofemoral Pain Syndrome: A Case-Control Study

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Abstract

Background: Patellofemoral pain syndrome (PFPS) is characterized by anterior knee pain due to arthralgia in the joint between the patella and femur. Many factors, including improper biomechanics and skeletal disorders, are associated with PFPS. The role of Vitamin D deficiency in the pathogenesis of patellar chondromalacia has been known for several years.

Objectives: The aim of the present study was to determine the prevalence of Vitamin D deficiency in young people with Patellofemoral Pain Syndrome (PFPS) and compare this with the prevalence in a healthy matched control group and to determine the correlation between the occurrence of biomechanical abnormalities and serum levels of 25(OH)D in patients with PFPS.

Methods: In this case-control study, 40 patients aged 15 to 40 years old with a diagnosis of PFPS, that had referred to the rehabilitation clinic of a university hospital in Tabriz, Iran, were selected as the case group and 40 normal subjects of the same age range were selected as the controls. Serum 25(OH)D levels were assessed, and a postural examination was performed on both groups, while the severity of knee pain, plain knee radiographs, and serum levels of calcium and phosphorous were assessed only in PFPS patients.

Results: Among the 80 participants, Vitamin D deficiency (cut-off level of 25(OH)D $\leq$ 20 ng/mL) was observed in 55 participants (68.75%), including 35 (87.5%) patients and 20 (50%) controls, with a statistically significant difference ($P < 0.001$). Females had a higher prevalence of Vitamin D deficiency than males, yet the difference was not statistically significant (71.21% versus 57.14%, $P = 0.348$). The serum levels of Vitamin D and pain severity were significantly and inversely related in the case group ($P = 0.005$). Clinical and imaging findings showed that 18 (45%) of the patients and two (5%) of the controls had abnormalities, such as genu varus, genu valgus, or patellar tracking, indicating a high coexistence of biomechanical deficits in PFPS ($P < 0.001$).

Conclusions: Severe and moderate Vitamin D deficiencies were more prevalent in young adults with PFPS than in normal adults. Knee pain severity and joint deformities were correlated with low levels of Vitamin D in the case group. Therefore, attention to diet, vitamin supplementations, and biomechanical correction are the mainstay treatment of PFPS.

Keywords: Arthralgia, 25-HydroxyVitamin D, Knee, Patellofemoral Pain Syndrome, Vitamin D, Deficiency

1. Background

Patellofemoral pain syndrome (PFPS), also known as patellofemoral arthralgia or chondromalacia, refers to pain in the joint between the patella and the femur. Arthralgia is the most common cause of knee pain in the younger population, especially in those, who participate in professional or recreational sports activities (1, 2).

Furthermore, PFPS is usually felt in activities, such as climbing up and down stairs or squatting and sitting for long periods of time. Although PFPS can follow an acute trauma to the patella or the cartilage under the kneecap, it usually has a gradual onset and chronic nature, due to joint overuse or overload (3, 4).

Other factors, such as the Q-angle increase (angle between the shaft of the femur and the tibia), biomechanical factors, such as muscle imbalance around the knee, patella medial tracking, patella lateral tracking, and joint curvature, such as genu varus (bow knee) and genu valgus (knock-knee), are the main underlying factors for PFPS. Other mechanical factors, such as flat foot, pes cavus (claw foot), lower-extremity alignment abnormalities, including outward twist of the tibia, femoral anteversion, and leg length discrepancy; muscle dysfunction, such as weakness

of vastus medialis oblique (VMO) in the quadriceps, iliotibial band (ITB) shortness, and hamstring muscles tightness have all been mentioned as PFPS risk factors (2).

Since Vitamin- D deficiency is an important cause of osteomalacia in the young population (1), it appears to be a predisposing factor for PFPS or chondromalacia. Vitamin D has long been known as an important factor for maintaining healthy bones and osteoblast metabolism, with a positive impact on bone mineralization (5). Circulating 25(OH)D level is the best marker of vitamin status, with a long half-time of 30 days (6). However, there is little consensus regarding the ideal 25(OH)D level. Nevertheless, many experts agree that 30 ng/mL represents an optimal 25(OH)D level and suggest that 20 ng/mL represents the lower limit of normal, while serum levels < 20 ng/mL are mostly defined as deficiency (6).

Severe Vitamin D deficiency in children leads to nutritional rickets and is associated with developmental delays and impaired growth (3, 7); thus, a low level of 1,25-DihydroxyVitamin D in patients causes a negative calcium balance and inhibits bone formation (8, 9). Therefore, adequate stores of Vitamin D are crucial for musculoskeletal health, so that peak bone mass achieved early in life is a predictor of osteoporosis risk in adulthood (7).

One of the latest studies in this regard showed that Vitamin D supplementation for six months reduced oxidative protein damage, decreased pain (VAS), improved quality of life, and improved grip strength and physical performance in osteoarthritis patients (10). Although studies have found no relationship between the exact range of Vitamin D deficiency and chronic pain, some studies suggested that levels < 25 ng/mL were associated with chronic knee pain, especially knee osteoarthritis, and increased sensitivity to pain (11).

PFPS is highly prevalent in teenagers, athletes, and other young adults with anterior knee pain. Furthermore, osteomalacia and patellar chondromalacia are among the known causes of PFPS and Vitamin D plays an essential role in the musculoskeletal system. In addition, there are a few relevant studies on this subject and further studies are warranted. Therefore, the current researchers decided to conduct a study to determine the prevalence of Vitamin- D deficiency among young adults with PFPS in Northwest Iran and compare the prevalence with that in a control group of individuals of similar age and gender. The study aimed at answering the following four questions: (1) Are there any significant differences in Vitamin D deficiency between cases and controls?; (2) Is there a relationship between serum levels of 25(OH)D and knee pain intensity in patients with PFPS?; (3) Is there a correlation between the occurrence of biomechanical abnormalities and serum levels of 25(OH)D in patients with PFPS?; and (4) Is there a relationship between the biomechanical abnormality type and serum levels of 25(OH)D?

2. Methods

The present case-control study included 40 patients and 40 healthy controls, selected through non-random convenience sampling.

Patients with anterior knee pain, who had referred to the Physical Medicine and rehabilitation clinic or referral from the Rheumatology clinic of Imam Reza University Hospital in Tabriz, Northwest of Iran, were recruited between April 2015 and April 2016.

Patients of both genders, aged 15 to 40 years old, who met at least two of the three following criteria were diagnosed as having Patellofemoral pain syndrome (PFPS) and were included in the study: (1) positive patellar grind test, (2) positive patellar compression test, or (3) knee joint line tenderness (1, 3). Subjects were excluded from the study if they had inflammatory joint diseases (rheumatoid arthritis, lupus erythematosus and so on), degenerative joint diseases (reduced tibiofemoral joint space), or other systemic diseases (brucellosis, septic arthritis, and so on).

After patients' selection, pain severity was quantified in the subjects using a 10-cm visual analog scale (VAS). Pain intensity was referred to as 0 to 10, in which 0 = no pain at all and 10 = the worst pain possible. Patients were asked to mark their pain level on the VAS scale.

2.1. Serum Vitamin D Level

Serum levels of Vitamin D, calcium, phosphorus, and alkaline phosphatase were measured. Blood samples were obtained from the study subjects after 12 hours of fasting. Serum 25-OH Vitamin D was measured within six hours of blood drawn by the electro chemiluminescent immunoassay (CLIA) technique (Roche Diagnostics company, Germany), run on a Cobas E411 auto analyzer at Imam Reza University hospital in Tabriz. These analyzer series provide suitable solutions for immunochemistry testing in low volume laboratories. This Vitamin D assay is intended for the quantitative determination of total 25-hydroxyVitamin D in human serum and plasma. Serum levels of Vitamin D were defined based on classifications proposed by the World Society of Endocrinology (12, 13): severe deficiency < 10 ng/mL, deficiency = 10 to 20 ng/mL, insufficiency = 20 to 40 ng/mL, sufficiency = 40 to 50 ng/mL, normal = 50 to 100 ng/mL, and near the toxic level > 100 ng/mL. In a broader sense, serum Vitamin D levels of < 20 ng/mL were considered as Vitamin D deficiency (14-16).

2.2. Biomechanical Abnormalities

Plain radiographs of the knee were taken in the anteroposterior, lateral, and patellar views and possible deformities (genu varus, genu valgus, patellar medial tracking, patellar lateral tracking, and so on) were assessed.

In genu varum alignment of the knee, the angle formed by lines through of the femur and tibia opens medially. In genu valgum alignment of the knee, the angle formed by lines through the femur and tibia opens laterally. In varus deformity, ankles are close together and in valgus deformity, knees are close together (17). The above mal alignments are diagnosed by physical examination and confirmed by knee anteroposterior X-ray.

Measurement of varus or valgus angle is performed by a radiological study of anatomical axes, yet in this study, there was no need for quantitative angle measurement and diagnosis of genu varum or valgus was enough, according to the above-mentioned criteria.

In general, the clinical perception is that in a normal patellofemoral joint, the patella lies centered in the femoral trochlear notch, equidistant from medial and lateral epicondyle. However, slight lateral deviation of the patella is considered normal. This deviation is no more than a few millimeters. Excessive deviation medially or more commonly laterally is known as medial or lateral tracking. A radiological study is necessary for the diagnosis of medial or lateral patellar tracking, so this item was diagnosed by using patellar view of the knee X-ray (17).

In addition, weakness of the quadriceps femoris muscle (mainly the VMO) and iliotibial band shortness were also studied by manual muscle testing and physical exam. The obtained data were recorded on a data collection form for each patient by one physiatrist. Other potential confounding factors, including the season of Vitamin D measurement, were recorded to determine the relationship between these variables and PFPS.

2.3. Sample Size

The study sample size was determined with regards to both the outcome of the study: "the prevalence of Vitamin D deficiency in young people with PFPS" and "the comparison of Vitamin D serum level in patients with PFPS and healthy controls"; the one that yielded a larger sample size was used. Considering the study prevalence outcome and according to the literature (15), with $\hat{p} = 0.59\%$, the type one error rate was set at $\alpha = 5\%$ to detect Vitamin D deficiency with the precision of 16%. The sample size was determined to be 36 subjects per group. By taking an expected drop-out rate of 10%, the total sample size was required to be 80 (40 in each group). The sample size for comparative purposes was also calculated regarding the mean (SD) of the Vitamin D serum level in cases and controls in the study by Khayyat, 2015 (18). Considering the power of 80% and confidence of 95% and regarding the possibility of 15% loss of samples, 36 subjects were estimated for each group. The mean (SD) serum level of Vitamin D was 21.10 (12.00) and 31.37 (16.35) for the case and control group, respectively. Eventually, the researchers obtained a larger estimated sample size; 40 subjects in each group.

2.4. Ethical Considerations

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences and informed consents were obtained from all study participants. The registry number of confirmation letter was 5/4/12044, and the Ethical code of this study was 91199. The blood tests and plain radiographs were performed as routine diagnostic procedures for patients with chronic knee pain, and no additional costs were incurred to them. The measurement of serum levels of Vitamin D in both case and control groups were paid for by the Research Center of Physical Medicine and Rehabilitation. Patients were excluded if they refused to undergo the tests.

2.5. Statistical Analysis

All the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software for Windows version 16.0 (SPSS Inc., Chicago, IL., USA). The studied demographic and clinical variables were summarized as means and standard deviations (SDs) for the continuous variables and frequencies and percentages for the categorical variables. Simple logistic regression analysis was conducted to identify potential predictors of Vitamin D deficiency (defined by a cutoff of $25(OH)D \leq 20$ ng/mL). The one-sample Kolmogorov-Smirnov test was performed to determine the frequency distribution of the studied variables. In case of non-normal data, median and interquartile range (IQR) was used to measure central tendency and dispersion of data and also non-parametric statistical methods were used in analytical statistics. Applying the appropriate transformation to ensure normal distribution was the researcher's alternative approach to deal with non-normal data.

The difference between group characteristics in terms of age (years), serum calcium level (mg/dL), serum phosphorus level (mg/dL), and serum alkaline phosphatase level (mg/dL) was declared using an independent sample t-test or the Mann-Whitney U test. Exact or Pearson Chi-square tests were used to verify differences between groups in terms of gender (F/M), season of Vitamin D measurement, and abnormality status (Y/N). The intergroup comparison of serum 25(OH)D concentrations was conducted using independent samples t-test. Simple logistic

regression was used to assess the impact of Vitamin D deficiency on PFPS. Finally, two-way Analysis of Variance (two-way ANOVA) was used to assess the intergroup effect of gender and season of Vitamin D measurement on serum 25(OH)D concentration. In case of significant intergroup differences, the Bonferroni post hoc test was conducted. Spearman rho correlation coefficient was used to investigate the influence of Vitamin D serum level on pain intensity. In this study, Graphpad Prism software GraphPad Prism Software for Windows Version 6.0 (GraphPad Software, Ladolla California, USA) was used to draw the graphs. P values of < 0.05 on the two-tailed test were considered statistically significant and table entries were obtained via 1000 bootstrap and Monte Carlo samples.

3. Results

In this study, 80 individuals aged 15 to 42 years (mean age, 28.18 $\pm$ 6.69; 33 females in each group [82.5%]) were recruited. Although females had a higher prevalence of Vitamin D deficiency than males, the difference was not statistically significant (71.21% versus 57.14%, P = 0.296).

The prevalence of severe Vitamin D deficiency (< 10 ng/mL), moderate deficiency (10 to 20 ng/mL), and insufficiency (20 to 40 ng/mL) in the total sample was 43.7%, 25.0%, and 20.0%, respectively. Furthermore, the results of logistic regression demonstrated that adults with biomechanical abnormalities were more prone to hypovitaminosis D than adults without biomechanical abnormalities (OR, 5.60; 95% CI, 1.57 to 63.93, P = 0.024). The Vitamin D deficiency prevalence within subgroups is presented in Table 1.

Radiographic and clinical assessments of the knee joint alignment resulted in 18 (45.0%) biomechanical abnormalities in the case group, including patellar medial tracking in six (15.0%), genu varum in two (5.0%), patellar lateral tracking in three (7.50%), vastus medialis weakness in two (5.0%), genu valgus in one (2.5%), patellar lateral tracking concomitant genu varus in one (2.5%), patellar lateral tracking concomitant genu valgus in one (2.5%), patellar lateral tracking with genu valgus in one knee and concomitant genu varus in the other knee (2.5%), and patellar medial tracking with genu varum and vastus medialis weakness in one (2.5%). In the control group, genu varus occurred in only two subjects (5.0%). In other words, biomechanical abnormalities were considerably more prevalent in the cases than in the controls (45.0% versus 5.0%, P < 0.001) (Tables 1 and 2).

In the case group, 25(OH)D levels were significantly less in a teenage girl with patellar lateral tracking and genu valgus in one knee, associated with genu varus in the other knee among all patients with biomechanical abnormalities (Figure 1).

The independent samples t-test results showed that the mean serum level of Vitamin D in the case group (11.57 $\pm$ 1.19 ng/mL) was significantly lower than that of the control group (23.04 $\pm$ 2.51 ng/mL) (P = 0.002). Among the total of 80 subjects, 55 (68.75%) were Vitamin D deficient (serum 25(OH)D level $\leq$ 20 ng/mL), including 35 (87.50%) in the case group and 20 (50.0%) in the control group (P = 0.002). On the other hand, subjects with PFPS were 6.82 times more likely to have Vitamin D deficiency than adults without PFPS (odds ratio [OR], 6.82; 95% confidence interval [CI], 2.79 - 38.98; P = 0.002). Severe deficiency of Vitamin D (< 10 ng/mL) was significantly more common in the case group (57.5%) than the control group (32.5%) (P = 0.002) (Table 3).

Results of the two-way ANOVA revealed a statistically significant interaction between the effects of gender and patellofemoral pain on Vitamin D serum levels (in logarithmic scale) ($f(1, 76) = 5.043$, P = 0.028); females with PFPS had significantly lower Vitamin D serum levels than healthy females (10.57 $\pm$ 7.41 versus 24.99 $\pm$ 16.64 ng/mL, P < 0.001).

There was a significant and inverse correlation between serum 25(OH)D concentration and pain intensity in the case group (Spearman rho correlation coefficient; r = - 0.398, P = 0.004). The results also indicated an interaction of season of measurement and PFPS on Vitamin D serum levels (in logarithmic scale) ($f(3, 72) = 2.766$, P = 0.048). In the spring, adults with PFPS had significantly lower Vitamin D serum levels (in logarithmic scale) than healthy adults (P < 0.001). However, among all the participants, serum Vitamin D levels were significantly lower during winter than in other seasons (Figure 2) (P = 0.029).

4. Discussion

The present study examined serum levels of Vitamin D in 80 subjects, including 40 patients with PFPS and 40 controls. Overall, severe Vitamin D deficiency was observed in 35 of the subjects (43.75%), including 23 cases (57.50%) and 12 controls (30.00%). Moderate Vitamin D deficiency was observed in 31 of the subjects (38.75%), including 16 cases (51.62%) and 15 controls (48.38%). The intergroup comparison showed that serum levels of Vitamin D differed significantly between the two groups (P = 0.001). On the larger scope, 68.75% of the young adult population had Vitamin D deficiency ($\leq$ 20 ng/mL).

In a study conducted at Boston Children's Hospital by Harvard University researchers, 24.1% of adolescents were Vitamin D deficient (serum 25(OH)D level $\leq$ 15 ng/mL, of whom 4.6% were severely Vitamin D deficient (25(OH)D

Table 1. Demographics and Clinical Findings of Participants (N = 80)

Characteristics	Value[a]	No. (%) of Subjects with Vitamin D Deficiency[b]	OR (95 % CI)
Age, y			0.963 (0.891 - 1.041)
Mean ± S.D	28.18 ± 6.69	NA	
Range	15.0 - 42.0		
Sex, No. (%)			
Female	66 (82.50)	47 (71.2)	1.85 (0.445 - 6.666)
Male	14 (17.50)	8 (57.5)	Reference group
BMI			0.957 (-4.963 - 0.228)
Mean ± SD	25.76 ± 1.92	NA	
Range	18.51 - 28.38		
Season, No. (%)			
Spring	27 (33.75)	16 (59.3)	Reference group
Summer	24 (30.00)	15 (62.5)	1.15 (0.360 - 3.497)
Autumn	13 (16.25)	10 (76.9)	2.30 (0.500 - 53.266)
Winter	16 (20.00)	14 (87.5)	4.81 (1.307 - 76.231)
Calcium serum level, mg/dcl			1.640 (0.578 - 5.778)
Mean ± SD	9.21 ± 0.90	NA	
Range	3.5 - 10.6		
Phosphorus serum level, mg/dcl			0.953 (0.431 - 2.852)
Mean ± SD	3.85 ± 0.67	NA	
Range	2.2 - 5.7		
Alkaline phosphatase serum level, mg/dcl			1.006 (0.997 - 1.029)
Mean ± SD	186.35 ± 22.12	NA	
Range	79.0 - 186.3		
Biomechanical abnormality, No. (%)			
Without biomechanical abnormality	60 (75.0)	37 (61.7)	Reference group
With biomechanical abnormality	20 (25.0)	18 (90.0)	5.595 (1.567 - 63.934)
Categories, No. (%)			
Patella medial tracking	6 (7.5)	6 (100.0)	
Genovarus	4 (5.0)	3 (75.0)	
Patella lateral tracking	3 (3.8)	3 (100.0)	
Vastus medialis oblique (VMO) weakness	2 (2.5)	2 (100.0)	
Genovalgus	1 (1.2)	1 (100.0)	
Patella lateral tracking + Genovarus	1 (1.2)	1 (100.0)	
Patella lateral tracking + Genovalgus	1 (1.2)	0 (0.0)	
Patella Lateral Tracking+ Genovalgus+ Genovarus in other knee	1 (1.2)	1 (100.0)	
Patella medial tracking + Genovarus + (VMO) weakness	1 (1.2)	1 (100.0)	

Abbreviations: CI, confidence interval; NA, no data applicable; OR, odds ratio.
[a]Data are presented as mean ± SD and range or frequency (percentage).
[b]Defined as a 25-hydroxyVitamin D level ≤ 20 ng/mL.

level ≤ 8 ng/mL). Using a broader definition, 42% of patients were Vitamin D insufficient (25(OH)D level ≤ 20 ng/mL) (19). Therefore, the prevalence of severe Vitamin D deficiency was still significantly higher in the present

Table 2. Demographics and Clinical Findings of Participants in the Study Groups[a]

Variables	Case (n = 40)	Control (n = 40)	P
Age, y	26.62 ± 6.27	29.72 ± 6.18	0.051[b]
Female, No. (%)	33 (82.5)	33 (82.5)	0.999[c]
Season, No. (%)			0.164[c]
Spring	12 (30.0)	15 (37.5)	
Summer	10 (25.0)	14 (35.0)	
Autumn	6 (15.0)	7 (17.5)	
Winter	12 (30.0)	4 (10.0)	
Calcium serum level, mg/dL	9.30 ± 0.51	9.10 ± 1.22	0.460[b]
phosphorus serum level, mg/dL	3.86 ± 0.62	3.84 ± 0.75	0.942[b]
Alkaline phosphatase serum level mg/dL	159 (75)	161 (64)	0.934[d]
Mechanical abnormalities, No. (%)	18 (45.0)	2 (5.0)	< 0.001[c]

[a] The data are presented as mean ± SD, median (IQR) or frequency (percentage %).
[b] Independent Samples t-test.
[c] Chi Square test.
[d] U-Mann Whitney test.

Table 3. Serum Level of Vitamin D and Amount of Deficiency Within and Between Study Groups[a]

	Vitamin D Serum Level (ng/mL), Mean ± SD (Range)	P Value	No. (%) of Subjects with Vitamin D Deficiency[b]	OR (95 % CI)
All Cases (n = 80)	17.30 ± 13.64 (3.0 - 54.5)	NA	55 (68.75)	NA
Case (n = 40)	11.57 ± 1.19 (3.0 - 35.5); 2.25 ± 0.64 (1.1 - 3.6)[d]	0.002[c]	35 (87.50)	6.82 (2.79 - 38.98)
Control (n = 40)	23.04 ± 2.51 (3.8 - 54.5); 2.85 ± 0.81 (1.3 - 4.0)[d]		20 (50.00)	Reference group

Abbreviations: CI, confidence interval; NA, no data applicable; OR, odds ratio.
[a] Data are presented as mean ± SD and range or frequency (percentage).
[b] Defined as a 25-hydroxyVitamin D level ≤ 20 ng/mL.
[c] Independent Samples t-test.
[d] To ensure normal distribution logarithmic transformation was applied.

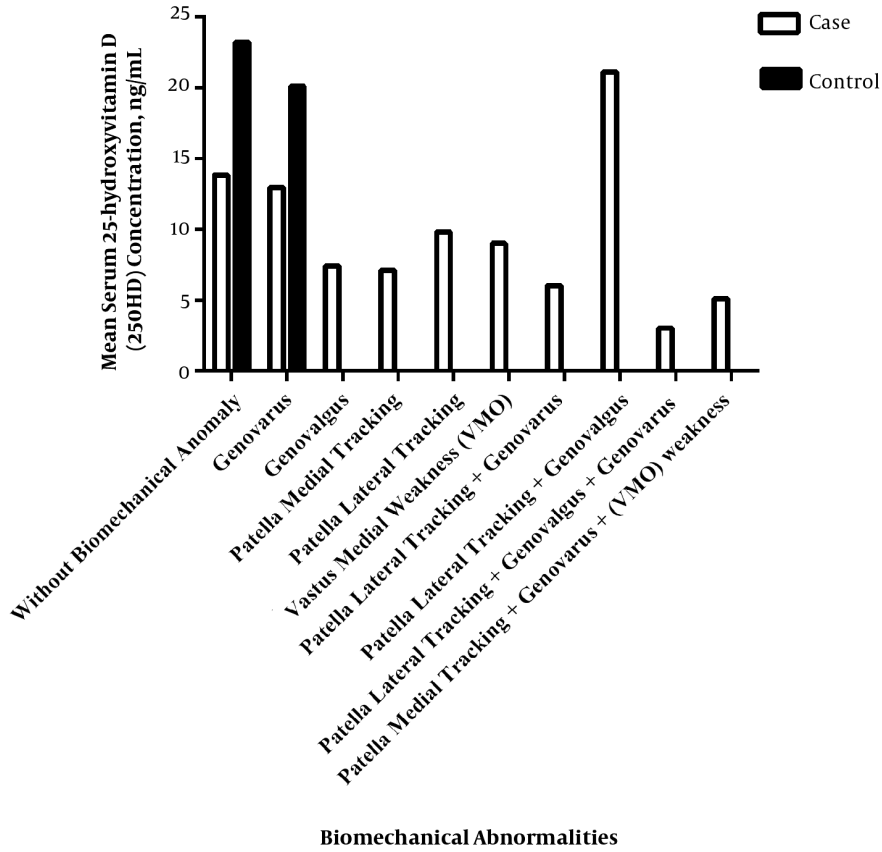
study than in similar studies, indicating the need to address diet and lifestyle in young adults in Iran.

In a study conducted by Muhairi et al. in the United Arab Emirates (UAE) on 15- to 18-year-old adolescents, 19.7% had deficiency (≤ 15 ng/mL), while 45.4% had insufficient levels of Vitamin D (≤ 20 ng/mL). The deficiency was more prevalent among females (18%) than males (10%) (7).

The prevalence of Vitamin D deficiency was higher in the current study than in the cited research. Nevertheless, it should be noted that Muhairi's cutoff point for deficiency was < 15 ng/mL, and using the definition of insufficiency (< 20 ng/mL) in that study, 45.4% were Vitamin D insufficient, a result close to the current study (50%) in a normal population. Differences in diet and, more importantly, lifestyle and culture in the examined region of the UAE and its hot climate and sun exposure throughout most seasons of the year compared to the cold climate of Azerbaijan (i.e., the region examined in the present study) could have affected the results.

In a study by Hovsepian et al. of 1111 healthy 20- to 80-year-old subjects in Isfahan, the mean 25(OH)D level was 21 ng/mL in males and 18 ng/mL in females. The prevalence of severe (< 10 ng/mL), moderate (10-20 ng/mL), and mild (20-30 ng/mL) deficiency was reportedly 26.9%, 23.6%, and 19.6%, respectively. The prevalence of Vitamin D deficiency in this sunny city was significantly higher in younger females as well as in the fall and winter (20). The authors attributed the remarkable Vitamin D deficiency, despite the region's favorable climate, to cultural reasons and the religious dress code (20). In the above-mentioned study, the prevalence of moderate to severe Vitamin D deficiency in the total population was about 50% of the 20- to 80-year-old population. In the present study, Vitamin D deficiency (< 20 ng/mL) had a prevalence of about 50% in the general population aged 15 to 42 years old, 87.5% in those with PFPS, and 68.75% overall. Nonetheless, the prevalence of severe deficiency and deficiency in the PFPS population in the current study was significantly higher than those in cited research.

The first obvious reason for this disparity was that the former study included only a population of healthy people, while the latter included a group of patients with chronic knee pain. The second reason was the difference in examined age ranges, since the former included mostly



Frequency of biomechanical abnormalities in study groups

Group	Without Biomechanical Anomaly	Genovarus	Genovalgus	Patella Medial Tracking	Patella Lateral Tracking	Vastus Medial Weakness (VMO)	More than one Biomechanical Anomaly
Control	38	2	0	0	0	0	0
Case	22	2	1	6	3	2	4

Figure 1. Legend: Participants' serum 25- hydroxyVitamin D (25OHD) concentration (ng/mL) by biomechanical abnormalities and group (n = 80)

people > 50 years of age, who usually take oral calcium and Vitamin D supplements, while young people do not use such supplements, thus, their serum Vitamin D level is more realistic and evidently lower. The third reason is related to different climates; while Hovsepian's study was conducted in central Iran, the present study was conducted in the coldest part of Northwest Iran.

Another study performed on 100 adolescents in Switzerland showed that a significant proportion of Caucasian teenagers were Vitamin D insufficient; however, this study failed to demonstrate the influence of low Vitamin D status on the bone mineral density of the lumbar spine and heel (6).

As expected, skeletal biomechanical disorders were far more common in the PFPS group than in the control group (18 versus two, respectively). The mean serum Vitamin D level in the group with biomechanical skeletal disorders was significantly lower than that of people without these disorders (mean, 9.94 ng/mL versus 19.76 ng/mL). In other words, this study found that subjects with biomechanical abnormalities were 5.60 times more likely to have Vitamin D deficiency than adults without these abnormalities.

However, the present study showed no significant correlations between biomechanical abnormality type, such as genu varum or genu valgus, and serum levels of Vitamin D in any of the subgroups. This result could be related to the small sample size of each subtype (one or two or six per abnormality), which hinders the ability to reach a meaningful difference.

The results of a cohort study on the relationship between moderate Vitamin D deficiency and knee and hip pain in 50- to 80-year-old patients showed that this defi-

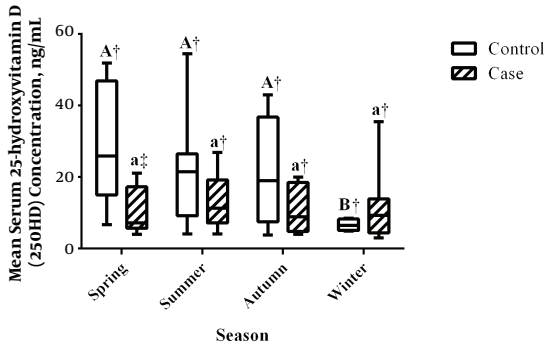


Figure 2. Legend: Participants' serum 25-hydroxyVitamin D (25OHD) concentration (ng/mL) by season and group (n = 80). Different uppercase letters on bars denote significant difference (P < 0.05, Bonferroni's test) based on intra-group analysis in the control group; Different lowercase letters on bars denote significant difference (P < 0.05, Bonferroni's test) based on intra-group analysis in the case group; different symbols on bars denote significant difference (P < 0.05, Bonferroni's test) based on inter-group analysis.

ciency could not predict the worsening or amelioration of pain within the next five years (21). However, the results of a systematic review performed in the US suggested that Vitamin D serum levels of < 25 ng/mL were associated with chronic knee pain, especially knee osteoarthritis and increased sensitivity to pain, mostly in the African-American race (11); however, many studies found no precise range of Vitamin D deficiency associated with chronic pain (20,21). Similar to previous studies, a significant and inverse relationship (P = 0.005 and Pearson's coefficient = -0.433) was observed between Vitamin D serum levels and pain severity in the present case group, so that as serum levels of Vitamin D decreased (particularly to < 10 ng/mL or severe deficiency), anterior knee pain severity increased considerably in the patients.

A study by Macfarlane et al. on the high incidence of generalized pain in a South Asian population and its strong association with Vitamin D confirms and explains the current findings (22). Macfarlane et al. suggested that widespread pain is common in the assessed South Asian population, partly due to the insufficient intake of Vitamin D. They noted that, although osteomalacia is the most known musculoskeletal disorder associated with Vitamin D deficiency, it often presents clinically as generalized skeletal pain. They also showed that low levels of Vitamin D and its association with generalized nonspecific pain was common among young females in this region and did not attribute this relationship to participation bias yet found it to be a real relationship even with minor changes. In their study, the risk factors associated with generalized pain, included psychological distress, lifestyle, insufficient exposure to sunlight, and low intake of Vitamin D in the daily diet (22). In other words, the low level of 25(OH)D may be a marker of individual low socioeconomic status.

In this regard, a study by Mabey et al. examined the role of Vitamin D on knee osteoarthritis at the molecular and cellular levels as well as in clinical presentations, and justified the function of Vitamin D deficiency in knee osteoarthritis and the mechanism of pain in PFPS by interrupting the bone remodeling process that is driven by subchondral bone osteoblasts and osteoclasts as well as the plurality of Vitamin D receptors in the knee joint (23). Vitamin D receptors are available in many tissues of the human body. Moreover, the effect of Vitamin D, as a selective immunosuppressor, is also mentioned in animal studies, in which Vitamin D prescription led to the prevention or inhibition of autoimmune diseases. In vivo studies indicated that appropriate levels of 1,25-dihydroxyVitamin D reduced the risk of inflammatory arthritis and rheumatic diseases (24, 25).

Finally, it should be noted that the initial treatment protocol of PFPS involves rehabilitation measures, such as the prescription of the proper orthosis, foot insoles, and kinesio taping techniques, for improving knee alignment or patellar tracking (26). Local injections of hyaluronic acid compounds and steroids, acupuncture, and even lateral retinaculum release or medial ligament tightening surgery have been proposed for those, who are resistant to conservative treatments (1, 3). Nevertheless, the principal treatment remains to correct the biomechanical disorders, strengthen the quadriceps femoris muscle, and improve hip muscle function (27).

4.1. Limitations and Suggestions

The present study was limited in some aspects. First, the two study groups did not perfectly match in terms of age. The mean age of the control group was higher than that of the case group (analysis type was modified with statistical methods). Second, this research was a single center versus multicenter study, and although extension or generalization of results to the entire population is less reliable yet the presence of a control group and comparing data with normal healthy matched people is the strong point of the study.

Therefore, future studies are recommended with a comprehensive multicenter survey in the country with similar number of male and female subjects in age-matched groups. In addition, further studies are suggested to evaluate the effect of medical therapy and Vitamin D on pain and function in these patients.

4.2. Conclusion

The current results suggest that the prevalence of severe and moderate Vitamin D deficiency in patients with

patellofemoral knee pain and healthy subjects was 87.5% and 50%, respectively, indicating a significant difference. Although females had a higher prevalence of Vitamin D deficiency than males, the difference was not statistically significant. Vitamin D deficiency in young adults with skeletal deformities differed considerably from that of young adults without biomechanical abnormalities, yet no relationship was seen with abnormality type. The degree of Vitamin D deficiency was the highest in winter in all subjects and in the spring in patients with PFPS. Pain severity was significantly and inversely related to serum Vitamin D levels in the case group. Therefore, it is recommended that a suitable diet and lifestyle of young adults be encouraged, musculoskeletal disorders be modified, and Vitamin D supplements be administered to target groups to effectively treat PFPS and the ensuing dysfunction.

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