Vitamin D Receptor Gene Polymorphism in Egyptian Patients with Knee Osteoarthritis

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Abstract: *Introduction*: Several polymorphisms have been implicated and associated with osteoarthritis (OA), including vitamin D receptor (VDR). VDR is present in many tissues, including chondrocytes. Vitamin D has been found to increase synthesis of proteoglycan *in vitro*, suggesting that it may affect the cartilage metabolism.

Objective: to investigate the frequency of the VDR gene polymorphism in Egyptian patients with knee OA, and compared them with controls.

Subjects and Methods: 200 patients with primary knee OA according to the American College of Rheumatology criteria and 200 matched controls who had no OA.

Clinical impact of OA were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire. VDR gene polymorphism *Bsm*I, *Apa*I, FokI and *Taq*I restriction fragment length polymorphisms (RFLPs) were determined by the polymerase chain reaction (PCR).

Results: The frequency of ApaI genotype and alleles did not differ significantly between the knee OA patients and the controls. However, three genotypes where significantly higher in knee OA patients than in controls: the bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI. Knee OA patients with osteophytes had significantly more frequent bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI. BsmI, TaqI and FokI genotypes or alleles did not show any association with the presence of joint space narrowing (JSN). Knee OA patients who had bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI are associated with significantly higher WOMAC scores.

Conclusion: The current study suggests that VDR gene polymorphism may be associated knee OA in the studied group of Egyptian patients. Association was mostly with severe clinical manifestation and osteophyte formation but not with JSN.

Keywords: Vitamin D receptor, Osteoarthritis, polymorphism.

INTRODUCTION

Osteoarthritis (OA) is a long-term chronic musculoskeletal disorder characterized by progressive degeneration of articular cartilage and joint space narrowing [1,2]. OA is the most common cause of physical disability in elderly population. Most of people had radiological evidence of OA by the age of 65 years [3].

OA is associated with diversity of both modifiable and non-modifiable risk factors, including the genetic predisposition [4]. Previous evidence indicates a genetic component to hand OA and knee OA ranging from 39% to 65% [5,6]. Family studies demonstrated that OA is transmitted in a clear Mendelian dominant pattern [7].

Several polymorphisms have been implicated and associated with OA, including vitamin D receptor (VDR). VDR is present in many tissues, including chondrocytes [8]. Vitamin D has been found to increase synthesis of proteoglycan *in vitro*, suggesting that it may affect the cartilage metabolism [9].

VDR gene is located on chromosome 12. Their distribution and frequency vary among ethnic groups [10].

The most frequently studied VDR gene polymorphisms were BsmI, ApaI (both in intron 8), and TaqI (in exon 9) [11]. The support for an association of *VDR* genotype with OA comes from studies that showed that serum levels of vitamin D are associated with the progression of knee and hip OA [12,13]. Moreover, Heidari *et al*. [14] found that vitamin D deficiency was associated with increased risk of OA and concluded that allelic variation in VDR gene may also be associated with OA.

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On the other hand, results from several studies suggested that the VDR gene polymorphisms are not important predictors of OA [15-17].

Liu *et al*. [18] conducted a meta-analysis to determine whether the polymorphisms in the VDR gene are associated with OA susceptibility and found that none of the VDR BsmI, TaqI, and ApaI gene polymorphisms were found to be significantly associated with the risk of OA. The relationship between of VDR gene polymorphisms and knee OA is still controversial.

To the best of our knowledge, the association between the VDR gene polymorphisms and knee OA has not been investigated in the Egyptian patients.

In this study, we investigated the frequency of the VDR gene BsmI, ApaI, FokI and TaqI restriction fragment length polymorphisms (RFLPs) in Egyptian patients with knee OA, and compared them with controls.

SUBJECTS AND METHODS

The present study was conducted on 200 consecutive patients with primary knee OA, they were recruited at the outpatient clinic of Rheumatology and Rehabilitation Department at Mansoura University Hospital, Egypt between January and September 2015. Patients were diagnosed according to the American College of Rheumatology (ACR) criteria [19]. The study also included 200 matched controls who had no OA. A written consent was obtained from each participant in this study after approval of the study protocol from Mansoura faculty of Medicine Institutional Review Board (MFM-IRB) (code: R/16.01.138). Patients with secondary knee OA previous arthroscopy or knee surgery were excluded from the study.

Patient assessment included history taking, physical examination, laboratory tests, and a review of medical records. Besides age and sex, we determined duration of OA and body mass index (BMI), smoking status and current drug intake. Most of our patients were on chondro-protective drugs and irregular courses of non-steroidal anti-inflammatory drugs (NSAIDs) and physiotherapy.

WOMAC Questionnaire

Clinical impact of OA were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire. The higher scores indicating greater symptom severity [20].

Radiological Examination

Weight-bearing antero-posterior and lateral semiflexed radiographs of both knees were done for all participants. A radiologist who was blinded from clinical data assessed the radiographs using the Kellgren Lawrence (KL) score [21] Grade1–2 changes according to KL were grouped as ‘early’ and stage 3–4 as ‘late’ radiological OA. Moreover, the presence of osteophytes and joint space narrowing (JSN) in the radiographs were also assessed.

Measurement of Serum 25-Hydroxyvitamin D3

At the same day of clinical evaluation, blood samples were obtained from all participants at 9 a.m. after over-night fast. All samples are processed in the laboratories of the Clinical Pathology Department of Mansoura Faculty of Medicine. Serum 25-hydroxyvitamin D3 (25-OHD) levels were measured by an ELISA DRG Instrument GmbH, Germany kit. Levels of vitamin D < 20 ng/ml were defined as deficiency meanwhile levels below 30ng/ml were defined as insufficiency [22].

Typing of Vitamin D Receptor Gene Polymorphism

Genomic DNA was extracted from whole-blood samples according to standard procedures (Intron Biotechnology, Korea). VDR genotype was determined by the polymerase chain reaction (PCR).

Genomic DNA was subjected to PCR analysis of the VDR gene using the following primers; ApaI (rs7975232) and BsmI (rs1544410); FokI (rs10735810) and TaqI (rs731236). Reaction conditions were carried out in thermocycler PTC-100 (Biorad, USA) with the following cycling parameters. The PCR conditions For ApaI and TaqI: digestion were initially 94 °C for 5 minutes then 35 cycles of 94 °C for 45 seconds, 60 °C for 45 seconds, and72 °C for 60 seconds and finally extension at 72 °C for 10 minutes. For BsmI digestion: the PCR conditions included initially 94 °C for 5 minutes then 32 cycles of 94 °C for 30 seconds, 51 °C for 30 seconds and 72 °C for 30 seconds and finally extension at 72 °C for 10 minutes. For FokI digestion, the PCR condition was 94 °C for 5 minutes then 35 cycles of 94 °C for 30 seconds, 63 °C for 30 seconds and 72 °C for 45 s and finally extension at 72 °C for 10 minutes. Then Ten microliter of PCR products were resolved in2% agarose gel to check the PCR products at 745 bp (ApaI, and TaqI), 512 bp (BsmI), and 267 bp (FokI). Restriction fragment length polymorphism

(RFLP) analysis was done using FastDigest ApaI, BsmI, TaqI and FokI (Fermentas, Germany). Genotypes were determined as follow; AA, Aa or aa for ApaI polymorphism; BB, Bb, bb for BsmI polymorphism. TT, Tt or tt for TaqI polymorphism; and FF, Ff or ff or FokI polymorphism.

Statistical Analysis

All statistical analyses were performed using SPSS for windows version 20.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ±standard deviation (SD), while categorical data were expressed in number and percentage. All continuous data were tested for normal distribution prior to any statistical calculations. The differences among patients with OA and controls were determined by independent samples t test for continuous data or chi-square test for categorical data. The association of osteophytes and JSN with the VDR alleles and genotypes in the OA patients was assessed using chi square test. Crude and adjusted ORs and their 95% confidence intervals (CIs) were also calculated for the association of the VDR with the osteophytes and JSN and for the comparison of the alleles and genotypes between patients and controls. The association between VDR genotype and alleles with the OA related features was assessed using the ANOVA test and the independent samples t test respectively. The Hardy–Weinberg equilibrium was done for the significance of association between the observed and expected number of the genotypes for a population Statistical significance was set at $p<0.05$.

RESULTS

The characteristics of the patients with OA and controls are demonstrated in Table **1**. Patients with knee OA had a significantly lower serum 25-OHD as compared to the controls. Moreover, patients with knee OA more frequently had 25-OHD deficiency and insufficiency than controls ($p<0.001$).

The distribution of VDR alleles and genotypes in knee OA patients and healthy controls was evaluated and demonstrated in Table **2**. The frequency of ApaI

Table 1: Demographic, Clinical and Laboratory Data in Knee OA Patients and Controls

	OA patients (n=200)	Controls (n=200)	p
	Mean ±SD	Mean ±SD	
Age (years)	60.1 ±8.3	58.9 ±8.9	0.141
Sex (n, %)			
Females	147, 73.5%	148, 74%	0.910
Males	53, 26.5%	52, 26%	
BMI (kg/m^2)	33.5 ±6.8	32.6 ±6.4	0.190
Disease duration (years)	6.8 ±3.5		
Smoking (n, %)	23, 11.5%	19, 9.5%	0.514
Womac disability score			
Pain	12.8 ±4.8		
Stiffness	4.7 ±2		
Functional	40.7 ±4.9		
Bilateralism (n, %)	126, 63%		
X-ray (n, %)			
Early	28, 14%		
Late	172, 86%		
25-OHD serum level ng/ml	22.8 ±10.4	31.4 ±11.4	<0.001
Vitamin D status (n, %)			
Deficiency	78, 39%	38, 19%	<0.001
Insufficiency	64, 32%	48, 24%	
Normal	58, 29%	114, 57%	

OA: osteoarthritis; BMI: body mass index; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; 25-OHD: 25-hydroxyvitamin D3; SD: standard deviation; OR = odds ratio.

Table 2: Distribution of VDR Alleles and Genotypes in Knee OA Patients and Healthy Controls

	OA patients N, %	Controls N, %	OR	95% CI	p
ApaI genotype					
AA	88, 44%	94, 47%	0.886	0.60 – 1.31	0.547
Aa	98, 49%	88, 44%	0.818	0.55 – 1.21	0.316
aa	14, 7%	18, 9%	0.761	0.37 – 1.58	0.461
ApaI alleles					
A	274, 68.5%	276, 69%	0.977	0.72 – 1.32	0.879
a	126, 31.5%	124, 31%			
BsmI genotype					
BB	16, 8%	53, 26.5%	0.24	0.13 – 0.44	<0.001
Bb	102, 51%	85, 42.5%	1.41	0.95 – 2.09	0.089
bb	82, 41%	62, 31%	1.24	0.83 – 1.85	0.037
BsmI alleles					
B	134, 33.5%	191, 47.8%	0.55	0.41 – 0.73	<0.001
b	266, 66.5%	209, 52.2%			
TaqI genotype					
TT	85, 42,5%	43, 21.5%	2.70	1.74 – 4.18	<0.001
Tt	97, 48.5%	106, 53%	0.84	0.56 – 1.24	0.368
tt	18, 9%	51, 25.5%	0.29	0.16 – 0.52	<0.001
TaqI alleles					
T	267, 66.8%	192, 48%	2.17	1.63 – 2.89	<0.001
t	133, 33.2%	208, 52%			
FokI genotype					
FF	132, 66%	83, 41.5%	2.74	1.82 – 4.11	<0.001
Ff	54, 27%	70, 35%	0.69	0.45 – 1.05	0.084
ff	14, 7%	47, 23.5%	0.25	0.13 – 0.46	<0.001
FokI alleles					
F	318, 79.5%	223, 55.8%	3.08	2.25 – 4.21	<0.001
f	82, 20.5%	177, 44.2%			

VDR: vitamin D receptor; OA: osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.

genotype and alleles did not differ significantly between the knee OA patients and the controls. However, BsmI, TaqI and FokI genotypes were significantly higher in knee OA patients than in controls: the bb genotype of BsmI was (41% versus 31% respectively), TT genotype of TaqI was (42.5% versus 21.5% respectively) and FF genotype of FokI was (66% versus 41.5% respectively). As regards the alleles frequencies between the knee OA patients and controls, b allele was significantly more frequent among the knee OA patients than in controls (66.5% versus 52.2%), T allele was more frequent in knee OA patients than in controls (42.5% versus 21.5%) and the F allele was more frequent in knee OA patients than in controls (66% versus 41.5%).

Knee OA patients with osteophytes had significantly more frequent bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI. The patients with osteophytes also had significantly more frequent b, T and F alleles of the BsmI, TaqI and FokI respectively than those without osteophytes (Table **3**). BsmI, TaqI and FokI genotypes or alleles did not show any association with the presence JSN (Table **4**).

As shown in Tables **5** and **6**, the duration of OA, WOMAC disability score and 25-OHD serum level did not show any association with the ApaI genotype or alleles. However, knee OA patients who had bb genotype of BsmI had significantly higher WOMAC pain, stiffness and functional scores and significantly

Table 3: Distribution of VDR Alleles and Genotypes in Knee OA Patients with and without Osteophytes

	Osteophytes (n=127) N, %	No osteophytes (n=73) N, %	OR	95% CI	p
ApaI genotype					
AA	58, 45.7%	32, 43.8%	1.08	0.60 – 1.92	0.802
Aa	59, 46.5%	35, 47.9%	0.942	0.53 – 1.68	0.840
aa	10, 7.8%	6, 8.2%	0.95	0.33 – 2.74	0.929
ApaI alleles					
A	175, 68.9%	99, 67.8%	0.954	0.33 – 2.74	0.821
a	79, 31.1%	47, 32.2%			
BsmI genotype					
BB	10, 7.8%	25, 34.2%	0.16	0.07 – 0.37	<0.001
Bb	71, 55.9%	32, 43.8%	1.62	0.91 – 2.90	0.100
bb	46, 36.2%	16, 21.9%	2.02	1.04 – 3.92	0.035
BsmI alleles					
B	91, 35.8%	82,56.2%	0.44	0.29 – 0.66	<0.001
b	163, 64.2%	64, 43.8%			
TaqI genotype					
TT	54, 42.5%	16, 21.9%	2.64	1.37 – 5.08	0.003
Tt	62, 48.8%	38, 52.1%	0.88	0.49 – 1.56	0.660
tt	11, 8.7%	19, 26%	0.27	0.12 – 0.61	<0.001
TaqI alleles					
T	170, 66.9%	70, 47.9%	2.20	1.45 – 3.33	<0.001
t	84, 33.1%	76, 52.1%			
FokI genotype					
FF	84, 66.1%	30, 41.1%	2.8	1.55 - 5.07	<0.001
Ff	34, 26.8%	27, 37%	0.62	0.34 – 1.15	0.131
ff	9, 7.1%	16, 21.9%	0.27	0.11 – 0.65	0.002
FokI alleles					
F	202, 79.5%	87, 59.6%	2.63	1.68 – 4.13	<0.001
f	52, 20.5%	59, 40.4%			

VDR: vitamin D receptor; OA: osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.

lower 25-OHD serum level than knee OA patients with Bb or BB genotypes. Also, OA patients who had b alleles of BsmI had significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25-OHD serum level than knee OA patients with B alleles (Table **6**).

Knee OA patients who had FF genotype of FokI had significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25-OHD serum level than OA patients with Ff or ff genotypes (Table **5**). Also, knee OA patients who had F alleles of FokI had significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25-OHD serum level than OA patients with f alleles (Table **6**).

Knee OA patients who had TT genotype of TaqI had significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25-OHD serum level than OA patients with Tt or tt genotypes (Table **5**). Also, knee OA patients who had T alleles of TaqI had significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25OHD serum level than OA patients with t alleles (Table **6**).

DISCUSSION

OA is a progressive multifactorial disease that not only leads to articular cartilage loss and joint space narrowing, but also to pain, loss of function and physical disability, thus greatly impairing quality of life.

Table 4: Distribution of VDR Alleles and Genotypes in Knee OA Patients with and without JSN

	JSN (n=91) N, %	No JSN (n=109) N, %	OR	95% CI	p
ApaI genotype					
AA	40, 44%	51, 46.8%	0.89	0.51 – 1.56	0.688
Aa	45, 49.5%	48, 44%	1.24	0.71 – 2.17	0.445
aa	6, 6.6%	10, 9.2%	0.70	0.24 - 2	0.503
ApaI alleles					
A	125, 68.7%	150, 68.8%	0.99	0.65 – 1.52	0.975
a	57, 31.3%	68, 31.2%			
BsmI genotype					
BB	12, 13.2%	25, 22.9%	0.51	0.24 – 1.08	0.077
Bb	46, 50.5%	45, 41.3%	1.45	0.83 – 2.55	0.190
bb	33, 36.3%	39, 35.8%	1.02	0.57 – 1.82	0.944
BsmI alleles					
B	70, 38.5%	95, 43.6%	0.81	0.54 – 1.21	0.301
b	112, 61.5%	123, 564%			
TaqI genotype					
TT	28, 30.8%	23, 21.1%	1.67	0.88 – 3.15	0.118
Tt	44, 48.4%	58, 53.2%	0.82	0.47 – 1.44	0.493
tt	19, 20.9	28, 25.7%	0.76	0.39 – 1.48	0.424
TaqI alleles					
T	100	104, 47.7%	1.34	0.9 – 1.98	0.149
t	82	114, 52.3%			
FokI genotype					
FF	47, 51.6%	45, 41.3%	1.52	0.87 – 2.66	0.143
Ff	24, 26.4%	41, 37.6%	0.59	0.32 – 1.09	0.091
ff	20, 22%	23, 21.1%	1.05	0.54 – 2.07	0.879
FokI alleles					
F	118, 64.8%	131, 60.1%	1.22	0.81 – 1.84	0.341
f	64, 35.2%	87, 39.9%			

VDR: vitamin D receptor; JSN: joint space narrowing OA: osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.

Table 5: Association between ApaI, BsmI, FokI and TaqI Genotypes and the Knee OA Related Features

	ApaIGenotype			
	AA	Aa	aa	p
Duration of OA	6.8 ±3.4	6.7 ±3.6	7.5 ±3.2	0.722
WOMAC Pain	13.1 ±4.9	12.4 ±4.9	13 ±4.5	0.638
WOMAC Stiffness	4.9 ±2.1	4.4 ±1.9	4.6 ±1.9	0.234
WOMAC Functional	41 ±4.9	40.4 ±4.9	40.2 ±4.9	0.679
25-OHD serum level	19.6 ±9.7	22.9 ±11	23.3 ±9.2	0.463

(Table 5). Continued.

	BsmI Genotype			
	BB	**Bb**	**bb**	**p**
Duration of OA	6.8 ±3.1	6.6 ±3.5	7 ±3.5	0.722
WOMAC Pain	11.1 ±4.4	12.7 ±4.8	14.2 ±5	0.025
WOMAC Stiffness	4.1 ±2.5	4.8 ±2	5.4 ±2	0.029
WOMAC Functional	39.1 ±5.8	39.9 ±4.7	41.8 ±4.8	0.014
25-OHD serum level	27.5±9.4	20.2 ±10.6	19.8 ±10.3	0.023
	FokI Genotype			
	FF	**Ff**	**ff**	**p**
Duration of OA	6.8 ±3.4	6.7 ±3.5	7.4 ±3.7	0.793
WOMAC Pain	13.9 ±4.8	12.8 ±4.7	10.2 ±4.9	0.015
WOMAC Stiffness	5.2 ±2	4.4 ±1.9	3.6 ±2.2	0.002
WOMAC Functional	41.9 ±4.9	39.6 ±5	39.2 ±3.9	0.005
25-OHD serum level	18.4 ±10.1	22 ±11	24.7±9.6	0.044
	TaqI Genotype			
	TT	**Tt**	**tt**	**p**
Duration of OA	7 ±3.4	6.4 ±3.5	7.7 ±3.5	0.259
WOMAC Pain	14.1 ±4.8	12.9 ±4.9	11.9 ±5	0.028
WOMAC Stiffness	5.1 ±2	4.8 ±2.1	3.7 ±2.2	0.034
WOMAC Functional	42.3 ±4.7	40.9 ±5.1	39.1 ±4.1	0.020
25-OHD serum level	19.1±10.5	23.7 ±10.1	26.2 ±11.2	0.003

OA: osteoarthritis; 25- OHD: 25-hydroxyvitamin D3;WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Table 6: Association between ApaI, BsmI, FokI and TaqI Alleles and the Knee OA Related Features

	ApaI Allele		
	A allele	**a allele**	**p**
Duration of OA	6.8 ±3.5	6.9 ±3.5	0.746
WOMAC Pain	12.9 ±4.9	12.6 ±4.7	0.567
WOMAC Stiffness	4.7 ±2.1	4.4 ±1.9	0.173
WOMAC Functional	40.8 ±4.9	40.4 ±4.9	0.426
25-OHD serum level	22.7 ±10.1	23.1 ±10.6	0.722
	BsmI Allele		
	B allele	**b allele**	**p**
Duration of OA	6.7 ±3.4	6.9 ±3.5	0.746
WOMAC Pain	12 ±4.7	13.4 ±4.9	0.007
WOMAC Stiffness	4.5 ±2.1	5 ±2	0.021
WOMAC Functional	40±5	41.1 ±4.8	0.034
25-OHD serum level	24.1 ±10.3	21.7 ±10.4	0.029

(Table 6). Continued

	FokIAllele		
	F allele	**f allele**	**p**
Duration of OA	6.7 ±3.5	6.9 ±3.6	0.638
WOMAC Pain	13.3 ±4.8	11.9 ±4.8	0.019
WOMAC Stiffness	4.8 ±2	4.2 ±2	0.028
WOMAC Functional	40.9 ±4.9	39.7 ±4.6	0.048
25-OHD serum level	20.9 ±10.3	23.5 ±10.5	0.043
	TaqIAllele		
	T allele	**t allele**	**p**
Duration of OA	6.8 ±3.5	6.7 ±3.5	0.877
WOMAC Pain	13.3 ±4.8	12.2 ±4.9	0.033
WOMAC Stiffness	5 ±2	4.3±2.1	0.013
WOMAC Functional	41.3 ±4.9	39.8 ±4.8	0.004
25-OHD serum level	21.1 ±10.3	24.2 ±10.3	0.005

OA: osteoarthritis; 25- OHD: 25-hydroxyvitamin D3; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

The knees, hips and hands joints are commonly affected [23].

VDR genes have been found to play a role in many diseases, including OA. This gene is located on chromosome 12q12-q14 [24]. However, the association of VDR gene polymorphism with OA remain debatable. The most frequently studied VDR gene polymorphisms in OA are: TaqI and BsmI and ApaI.

The major finding of our study is that three genotypes were significantly higher in OA patients than in controls: the bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI while ApaI genotype and alleles did not differ significantly between the knee OA patients and the controls. Consequently, b allele of BsmI genotype, T allele of TaqI genotype and F allele of FokI genotype were more frequent in knee OA patients than in controls.

Additionally, knee OA patients who had bb genotype of BsmI, TT genotype of TaqI and FF genotype of FokI are associated with significantly higher WOMAC pain, stiffness and functional scores and significantly lower 25-OHD serum level.

The positive association between VDR gene polymorphism and susceptibility to OA was reported by Keen *et al.* [25] who showed that postmenopausal British English women with TT or Tt genotype had an increased risk of knee OA compared with women with tt genotype.

Also, in The Netherlands Uitterlinden *et al.* [26] found that VDR gene polymorphism is associated with OA of the knee. They demonstrated that the`baT' haplotype was over-represented in OA.

Moreover, other studies reported that allelic variations of the VDR gene were associated with the severity of spinal degenerative disease [27,28]. Solovieva *et al*. [29] suggested an association between the *VDR* ApaI and TaqI polymorphisms and symmetrical hand OA in Finnish women. Moreover, Granchi *et al*. [30] found the homozygous bb is more frequent in patients with secondary OA of to hip.

On the other hand, Aerssens *et al.* [31] and Tamai *et al.* [32] did not support the hypothesis that VDR gene polymorphism are associated with OA, they did not find any association between hip OA and VDR gene polymorphisms in Belgian and Japanese women, respectively. Also, Baldwin *et al*. [17] failed to find any association between VDR gene with knee OA in American patients.

Furthermore, Huang *et al*. [16] 2000 did not find any association between VDR gene polymorphism (BsmI, ApaI and TaqI) and knee OA in Japanese women.

In the study of Muraki *et al.* [33], knee pain was significantly associated with ff genotype compared with the FF genotype. They also reported no significant associations of ApaI polymorphisms with knee pain.

The meta-analyses of Zhu *et al*. [34] showed no significant associations between VDR TaqI or BsmI polymorphism and OA in different races. They also found a significant association between VDR FokI(ff)

genotype and OA in all studied groups. Moreover, they found significant associations between VDR ApaI polymorphisms and OA only in the Asian patients. They suggest that VDR gene polymorphisms may be involved in the pathogenesis of OA aetiology in the Asian population.

However, Lee *et al*.[35] did not find any significant associations between VDR (TaqI, BsmI, ApaI) polymorphisms and OA among European or Asian patients. The cause may be due to the clinical heterogeneity of their meta-analysis as they include studies on lumbar disc degeneration, stenosis in which pathologic mechanisms were not the same as that of OA. Their results were also supported by those of Liu *et al*. [18].

These controversy results among studies, may be due to different factors, including the races, genetic, and age of the studied population. The mechanisms of OA may be not the same at different sites.

Moreover, we observed that 25-OHD serum levels were significantly lower in OA patients than controls. These results are consistent with other studies which reported the increased prevalence of vitamin D deficiency in OA patients. It was suggested that Vitamin D plays an important role in bone mineralization, remodeling, and maintenance and therefore its deficiency may be implicated in the pathogenesis of OA [14,22].

Radiographic osteoarthritis (ROA) was defined in our study using *the Kellgren score* [21], which assess two characteristics (osteophytes and joint space narrowing). Osteophytes are osseous and cartilaginous neoplastic protrusions forming mostly at the margin of osteoarthritic joints while JSN is thought to be due to degeneration of cartilage.

In the present study, knee OA patients with osteophytes had significantly more frequent bb genotype of BsmI, TT genotype of TaqI and had FF genotype of FokI and also had significantly more frequent b, T and F alleles of the BsmI, TaqI and FokI respectively than patients without. On the other hand, no significant association between VDR gene polymorphism and JSN.

This interesting finding suggests an important role of the VDR gene in relation to the osteophytes formation rather than cartilage loss.

Similarly, Griffith *et al*. [36] found an association of VDR genotype with ROA of the knee in the United Kingdom.

This association between VDR gene polymorphisms and osteophytes was observed also in Britons as well as in Australians and Finns [25,27,28].

Uitterlinden and colleagues [15,26] demonstrated that VDR gene polymorphism (*T* allele, *a* allele) is related to osteophyte formation, not to JSN, in subjects with knee OA in The Netherlands study. Moreover, Muraki *et al*.[33] found that a FokI polymorphism of the VDR was significantly associated with the presence of osteophytes rather than JSN.

While, Solovieva *et al*. [29] and Valdes *et al*. [37] did not find any association between TaqI polymorphism and knee OA (osteophytes or JSN).

CONCLUSION

The current suggests that VDR gene polymorphism may be associated knee OA in the studied group of Egyptian patients. Association was mostly with severe clinical manifestation and osteophyte formation but not with JSN.

RECOMMENDATION

Further studies is required to study the association between the VDR gene and OA in different sites in a large group of OA patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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