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Missense FokI variant in the vitamin D receptor gene in primary knee osteoarthritis patients in south Indian population

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ABSTRACT

Osteoarthritis (OA) is a common and multifactorial skeletal disabling disease in the adult/elder population. Vitamin D was involved with multiple diseases including OA. Based on the prior studies, the single nucleotide polymorphism in the vitamin D receptor (VDR) gene might be a risk factor for the development of autoimmune diseases. However, we aimed to investigate whether FokI polymorphism and serum vitamin D levels are in association with primary knee OA in south Indian population. In this case–control study we have selected 100 OA cases and 100 healthy controls from Kamineni Hospitals in Telangana and India. The collected blood samples were used for estimation of serum vitamin D levels and isolation of genomic DNA. PCR was carried out with specific primers. The FokI enzyme was used to perform RFLP and followed by electrophoresis and analysis. The clinical data has been calculated separately between the OA cases and controls. The biochemical analysis performed with ELISA method revealed that 44% of cases were vitamin D deficient, 28% had in sufficient vitamin D levels and 28% had sufficient vitamin D levels. We have performed the statistical analysis between the OA cases and controls and we could not find any significant association either with allele and genotype frequencies ($p > 0.05$). The gender association analysis also failed to disclose the significant difference between males and females ($p > 0.05$). The results of our present study concludethat genetic missense variant of FokI in VDR gene does not associate with primary knee OA in the south Indian population.

1. Introduction

Osteoarthritis (OA) is a common chronic and multifactorial etiology with a group of mechanical abnormalities involving degradation of joints and subchondral bone (He et al., 2014). The prevalence of OA varies widely between studies and depends on the study population and increases with age with osteoarthritic changes uncommon under the age of 40, but seen in most people over the age of 70. Obesity and overweight are the major factors that became rapidly growing threat to worldwide health and the co morbidities include hypertension, type2 diabetes, stroke and cardiovascular diseases.

The knee, hip and hand are most affected by the disease (Van Schoor et al., 2015). Knee OA is the commonest degenerative arthritis. Knee OA is a mechanically driven disease and it is suggested that medial tibiofemoral knee-joint load increases with pharmacologic pain relief, indicating that it may be positively associated with disease progression. The underlying mechanisms are still unknown; a genetic component has been established by population studies. Genetic factors contribute to the development of OA the heritability has been estimated at 48%–65% (Ai et al., 2014). The World Health Organization Scientific Group on Rheumatic Diseases estimates that among those people who are 60 or older, the incidence rate of OA is up to 1 in 10 (Pereira et al., 2011). Age, sex, obesity, ethnicity and profession are the multiple risk factors and well recognized environmental factors, genetic variations have also been found to be strong determinants (Cai et al., 2014). The involvement of vitamin D in bone and cartilage metabolisms could explain why alterations in vitamin D homeostasis are associated to several pathological conditions of knee cartilage and intervertebral disc tissue, in particular OA and disc degeneration linked pathologies (Colombini et al., 2013). Single nucleotide polymorphisms (SNPs) are single base variations between genomes, useful for genome-wide

Abbreviations: OA, Osteoarthritis; VDR, Vitamin D Receptor; PCR, Polymerase chain reaction; RFLP, Restriction Fragment Length Polymorphism; ELISA, Enzyme Linked Immunosorbent Assay; SNP, Single Nucleotide Polymorphism; SNPs, Single Nucleotide Polymorphisms; K/L Grade, Kellgren/Lawrence Grade; 25OHVD, 25-hydroxyvitamin D; OR, Odds Ratio; CIs, Class Intervals.

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mapping and study of disease genes. SNP is a useful marker for diseases in haplotype-based association studies and in linkage disequilibrium analysis (Khan et al., 2016). Ng and Henikoff (2001) have predicted the effect of SNPs on protein function based on sequence homology methods and many polymorphisms in vitamin D Receptor (VDR) were identified. VDR gene covers 105 kb, with an extensive promoter region capable of generating multiple tissue-specific transcripts (Colombini et al., 2013). VDR harbors several known functional polymorphisms and some of these have been investigated (He and Wang, 2015). Several studies investigated the role of VDR (FokI)/rs2228570 polymorphism in the susceptibility to common diseases such as T2DM, osteoporosis and OA. Based on the prior studies, we have carried out our study to investigate whether FokI polymorphism and serum vitamin D levels are in association with south Indian population.

2. Materials and methods

2.1. Ethics

Ethical approval for this current research was obtained from the Institutional Ethics Committee, Kamineni Hospitals, Hyderabad, India. Data and blood collection from study individuals were collected after taking their informed consent.

2.2. Patient recruitment

The hospital based case–control study has been carried out in one hundred (n = 100) south Indian OA patients who were clinically diagnosed and radiologically confirmed with primary knee OA and 100 healthy controls were arbitrarily selected from the routine master health checkup examination who did not have any complaints of OA or autoimmune diseases. The selection, inclusion and exclusion criteria of cases and controls have been published in our earlier paper (Poornima et al., 2015). All the patients were recruited from Department of Orthopedics, Kamineni hospitals, Hyderabad, India. The age range of the patients was 25–60 years (42.4 ± 8.1) and the age range of controls was 26–62 (42.1 ± 7.9). Personal, medical and family histories were documented in a well-designed proforma.

2.3. OA score

The radiographic image and clinical information were used for the Kellgren/Lawrence (K/L) score (0–4 scale). K/L system is a method of classifying the severity of knee osteoarthritis using five grades (Kellgren and Lawrence, 1957)

1. Grade 0: No radiographic features of Osteoarthritis
2. Grade 1: Doubtful joint space narrowing and possible osteophytic lipping.
3. Grade 2: Definite osteophytes and possible joint space narrowing on anteroposterior weight bearing radiograph
4. Grade 3: Multiple osteophytes, definite joint space narrowing, sclerosispossible bony deformity.
6. Grade 4: Large osteophytes marked joint space narrowing, severe sclerosis and definitely bony deformity.

2.4. Investigation of FokI polymorphism

Four milliliters of the peripheral blood was collected and 2 mL of the serum was used for measurement of 25-hydroxyvitamin D (25 OH VD) levels, by ELISA method using IDA kit manufactured by Immunodiagnostic Systems Ltd., UK. Individuals were categorized as deficient (<20 ng/ml), insufficient (20–30 ng/ml), sufficient (30–100 ng/ml) and potential toxic (>100 ng/ml). The remaining 2 mL of the blood was used for genomic DNA extraction using salting out technique, the standard method used in the Department of Genetics and Molecular Medicine, Kamineni hospitals (Khan et al., 2015). The genomic DNA was quantified with NanoDrop and stored at −80 °C until further use. Genotyping determination for fok1 polymorphism in the VDR gene, located in exon 2 was carried out with polymerase chain reaction and restriction fragment length polymorphism performed with 2% agarose gel electrophoresis.

Table 1
Demographic details of the patients and controls.

Characters	Cases ($n = 100$)	Controls ($n = 100$)	p value
Age (years)	42.41 ± 8.11	42.17 ± 7.98	0.87
Sex: (M:F)	32:68	31:69	0.88
Height (cm)	156.63 ± 2.68	155.46 ± 2.45	0.37
Weight (kg)	76.97 ± 8.44	69.78 ± 7.87	0.001
BMI (kg/m^2)	31.38 ± 3.4	25.89 ± 2.67	0.01
Age of onset	39.52 ± 6.89	NA	NA
Family history of OA	28 (28%)	NA	NA
History of HTN	50 (50%)	15 (15%)	<0.01
History of T2DM	38 (38%)	14 (14%)	<0.01
History of thyroid dysfunction	39 (39%)	22 (22%)	<0.01

2.5. Statistical Analysis

The association of genotype in OA was examined by odds ratio analysis with 95% confidence interval using Openepi software (Khan et al., 2014). The allele frequencies of VDR gene were tested to determine whether they were in accordance with HWE (Hardy–Weinberg equilibrium). Clinical data are expressed as mean ± standard deviation. Data analysed by ANOVA. An independent t-test was used to test the case and controls. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Demographic details of the patients and controls

The clinical features of cases and controls have been described in Table 1. The mean age of the cases was 42.4 ± 8.1 and controls was 42.1 ± 7.9, which are similar ($p = 0.87$). The clinical features such as age, gender and height were also similar between cases and controls. The weight and BMI of cases was significantly greater ($p < 0.05$). Comorbid factors hypertension, thyroid dysfunction and T2DM were more in the cases compared to controls ($p < 0.05$). In this study, 28% of the cases had positive family history of OA.

3.2. Biochemical analysis

The serum concentration of 25 OH VD was measured using ELISA method. This test was performed for the identification of regulation of bone metabolism and calcium homeostasis effects as a deficiency or excess of vitamin D. We have measured the vitamin D levels in knee osteoarthritic cases. The results of our study showed that 44% of cases were vitamin D deficient, 28% had in sufficient vitamin D levels and 28% had sufficient vitamin D levels.

Table 2
Genotype and allele distribution of VDR-*Fok1* gene polymorphism.

Genotype	OA cases	Controls	OR 95% CI	p value
CC	95 (95%)	99 (99%)	1.00	
CT	05(5%)	01 (1%)	5.2 (0.5–45.40)	0.13
TT	0 (0%)	0(0%)	3.1 (0.61–15.87)	0.16
C	195 (0.975)	199 (0.995)	1.00	
T	05 (0.025)	01 (0.005)	5.1 (0.5–44.5)	0.13