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VIT-D receptor gene polymorphism and its relation to lumbar disc degeneration

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Abstract
Introduction: Low back ache (LBP) or lumbago is one of the commonest patient complaints encountered in clinical practice with about (80%). Lumbar disc degeneration (LDD) is the fundamental driver of low back pain. Vitamin D is considered as a hormone that is responsible for calcium homeostasis and bone mineralization. Vitamin D receptor (VDR) gene is the first reported gene possibly connected with LDD risks. The present study was conducted to pursue the potential correlation between common allelic variants in vitamin D receptor locus and degeneration of the lumbar spine.
Materials & Method: This was a Prospective Case Control Study performed with 30 cases with related complaints and findings, a similar number of age and sex matched controls were included. A detailed performa was prepared and maintained. Blood samples were collected in a standard manner and processed for determination of genotypes of Vit D receptor. DNA processing was done followed by alphabetical designation of the genomes. The findings were arranged as quantitative data and was represented as their mean ± SD while categorical data was expressed in numbers and percentage.
Results: Apart from demographic and other findings it was found that the prevalence of wild genotype (CC) was more prevalent in cases as compared to controls. Prevalence of wild allele (C) was more prevalent in cases as compared to controls and the study shows that C allele was higher in cases with LDD, however both the differences were statistically not significant. The cases with CC genotype, developed LDD at a significantly younger age than cases with TT genotype.
Conclusion: The present study provided useful information regarding common allelic variations in vitamin D receptor locus and degeneration of the lumbar spine. Our study concludes that VDR expression could be used as an important marker for monitoring lumbar disc degeneration in cases with lower back pain.

Keywords: Low back ache (LBP), lumbar disc degeneration (LDD), Vit D receptor (VDR), gene polymorphism

Introduction
Low back ache (LBP) or lumbago is one of the commonest patient complaints encountered in clinical practice, having significant economic consequences to the affected patient, especially young employed individuals thereby leading to loss of national economy by loss of labour working days. The disease is particularly common in manual workers, weight lifters and IT professionals. The evolution of mammals from quadrupeds to bipeds resulted in a shift in the center of gravity and subsequent mechanical stress on the lumbar spine. The occurrence of low back ache can be probably attributed to this evolution in the basic posture of human beings.
Eighty percent (80%) of the adult population suffers from LBP at some time in their lives [1]. Around 10% become chronically disabled [1]. Patients with this disease may also present with sciatic symptoms. The causes of lumbago are many and include abnormalities of the lumbo-sacral spine or those related to the soft tissue surrounding the lumbo-sacral spine [2].
Lumbar disc degeneration (LDD) is the fundamental driver of low back pain [3]. Degenerated discs happen in 40% of people under 30 years of age and more prominent than 90% of those more than 50 (4). The risk factors for LDD incorporate family history, lumbar load and work stack, so the occurrence of LDD is much more in developing states.

The etiology of DD was only ascribed to the build-up of environmental factors, injury, and way of life, smoking, atherosclerosis, and disc degeneration with aging [5]. Nevertheless, recent researches have shown that the impact of these components is moderate in DD, fortifying the conviction of hereditary contribution in the causes of the ailment [6].

Vitamin D is considered as a hormone that is responsible for calcium homeostasis and bone mineralization [7]. Vitamin D biological activities are regulated by a high-affinity receptor that works as a transcription factor activated by ligands.

Vitamin D receptor (VDR) gene is the first reported gene possibly connected with LDD risks [8]. It is found on human chromosome 12 [12q12-q14]; its length is 100 kb, with more than 100 restriction cutting sites [9]. VDR is one of the steroid super-families of nuclear receptor, which mostly manages the transcriptional activity of 1,25-dihydroxyvitamin D3 [active metabolite of vitamin D] [10]. The VDR FokI polymorphism is an autonomous polymorphic site situated in exon 2 and prompts an alternate translation initiation site, prompting the development of a longer VDR isoform, expected to be less active [7].

Previous studies examined the relationship of polymorphisms in VDR gene Fok1 polymorphism with LDD [11]. However, their data are conflicting in different population groups.

The present study was thus conducted to pursue the potential correlation between common allelic variants in vitamin D receptor locus and degeneration of the lumbar spine.

Materials & Methodology

This study was a hospital based Case Control study where we studied a total of 30 patients, who visited the Orthopaedics OPD at Sassoon General Hospital attached to BJGMC, Pune from October 2015 to July 2017 with complaints of Low Back Pain (LBP) and MRI scans suggestive of Lumbar Disc Degeneration. A similar number of age and sex matched controls were included. The cases and controls were included in the study after proper informed consent in local language.

Methodology

Detailed history and thorough clinical examination was performed. The findings were recorded in the proforma. The recorded data included demographics, duration and type of pain and other associated symptoms. MRI was performed in all cases. Degeneration is graded as

- 0 : No single change
- 1: Slight decrease in signal intensity in nucleus pulposus
- 2: Distinct decrease in signal intensity in nucleus pulposus with normal disc height.
- 3: Sever decrease in signal intensity in nucleus pulposus with disc space narrowing.

Data Collection

Determination of Genotypes

Blood sample (2 ml) was collected from the ante- cubital vein in (EDTA) tubes from cases and controls. Genomic DNA was extracted from white blood cells according to the procedure of the DNA assay Midi kit (Qiagen, Duesseldorf, Germany). Polymerase chain reaction and restriction fragment length polymorphism (PCRRFLP) methods was applied to detect the FokI polymorphism of VDR. Genomic DNA was amplified using PCR. At first DNA was denatured at 95 ºC for 5 minutes. Standard PCR conditions was as follows: 94 ºC for 1 minute, annealing temperature of 63 ºC for 1 minute and 72º C for 2 minutes for 35 cycles and finally 96 ºC for 1 minute and 72 ºC for 5 minutes. The FokI polymorphism of VDR was studied using previously tested primers. The resulting 265 base pairs DNA fragment was digested with FokI restriction enzyme generating two fragments of 196 and 69 base pairs only in presence of the f allele (T). DNA fragments were separated on polyacrylamide gel. Randomly chosen sample's gel results were confirmed by DNA sequencing. Genotypes were designated by a lowercase letter (f allele, T nucleotide, mutated) for the presence of the restriction site and by a capital letter (F allele, C nucleotide, wild -type) for its absence.

Statistical Analysis

The quantitative data was represented as their mean ± SD while categorical data was expressed in numbers and percentage. Association of Vitamin D receptor gene polymorphism with lumbar disc degeneration was computed using chi-square test. All analysis was carried out by using SPSS software version 21.

Inclusion Criteria

Patient having all of the following criteria were included:

1. Presence of backpain more than 3 months
2. MRI showing at least one lumbar disc degeneration.
3. Age group between 30 to 60 years.

Exclusion Criteria

1. Age <30 or >60 years.
2. Presence of any neuro-deficit.

Results

Table 1: Distribution of cases as per Age

Age Group (years)	Cases	Controls
< 30	5	5
31-50	17	17
> 50	8	8
Total	30	30
p- value 1.0		

Mean age of cases was 45.14 years with over half of them (56.7%) were between 31-50 years of age. Similar number of age matched controls were also taken.

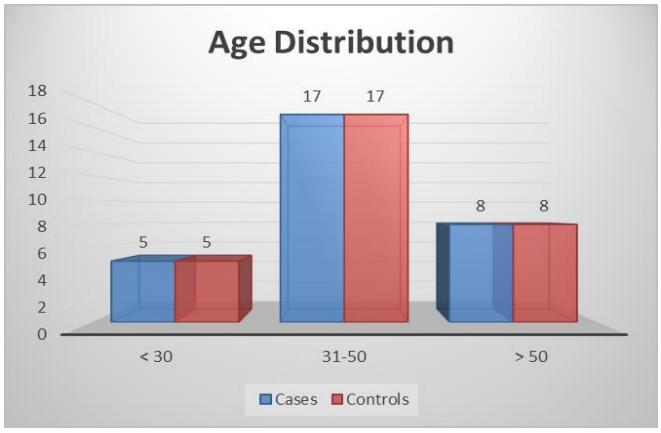


Table 2: Distribution of cases as per Gender

Sex	Cases	Controls
Female	13	13
Male	17	17
Total	30	30
p- value 1.0		

A slight male predominance was observed among study cases (56.7% males to 43.3% females). Similar number of gender matched controls were also taken.

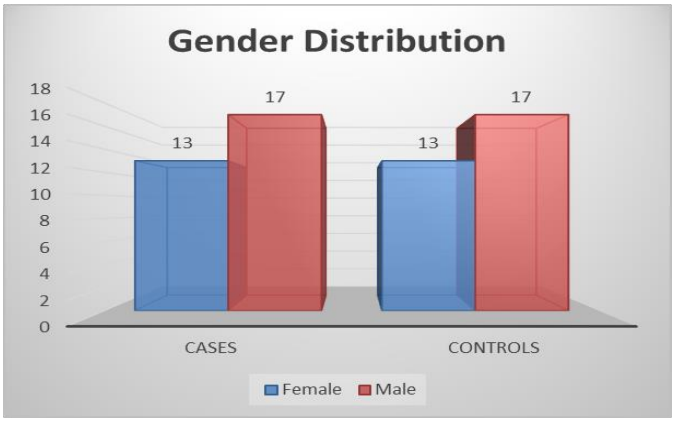


Table 3: Distribution of cases as per Duration of symptoms

Duration of Symptoms	Cases	%
≤ 12 months	24	80.0%
13-24 months	5	16.7%
> 24 months	1	3.3%
Total	30	100.0%
Mean duration: 7.46 ± 4.73		

Duration of symptoms among most cases (80%) was less than 12 months. Duration of symptoms more than 2 years was seen only in1case.

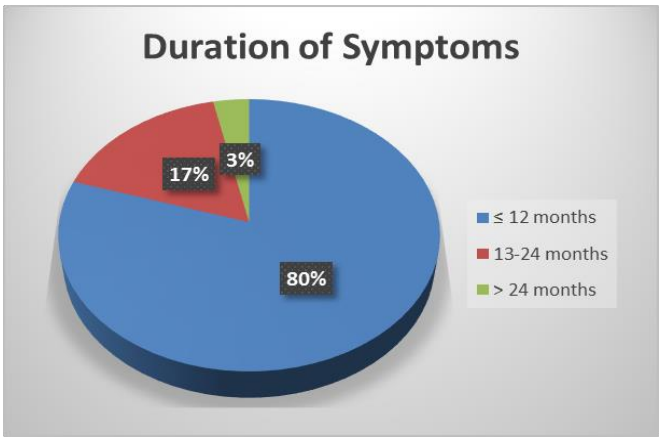


Table 4: Distribution of cases as per Type of symptoms

Symptoms	Cases	%
Radiating Pain	19	63.3%
Tingling/ Numbness	10	33.3%
Loss of Lumbar Lordosis	22	73.3%
Loss of Bowel/ Bladder Control	4	13.3%

Most common presenting symptom was loss of lumbar lordosis (73.3%) followed by radiating back pain (63.3%), tingling/ numbness (33.3%) and loss of bowel/ bladder control (13.3%).

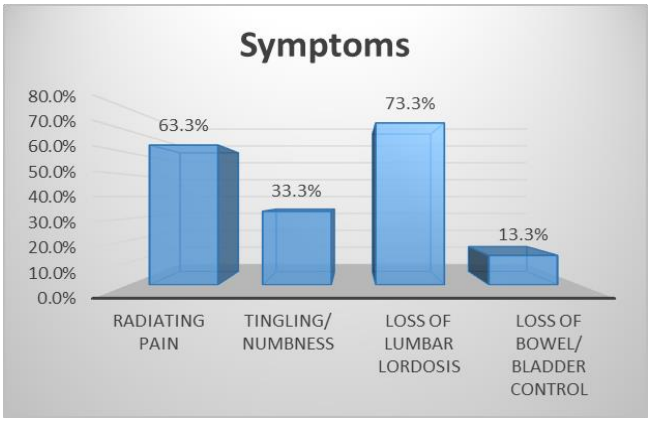


Table 5: Distribution of cases as per Lumbar disc degeneration severity

LDD Severity (MRI)	Cases	%
I	17	56.7%
II	8	26.7%
III	5	16.7%
Total	30	100.0%

As per MRI report, Slight decrease in signal intensity in nucleus pulposus was seen in 56.7% cases while in 26.7% and 16.7% cases there was a distinct and severe decrease in signal intensity.

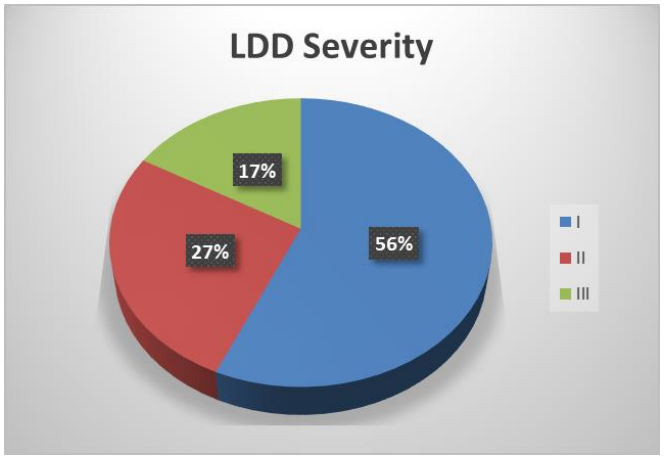


Table 6: Distribution of cases as per prevalence of FokI genotype among cases

FokI Genotype	Cases	%
TT	12	40.0%
TC	11	36.7%
CC	7	23.3%
Total	30	100.0%

Among cases, prevalence of mutated genotype (TT) was 40% while that of wild genotype (CC) as 23.3%.

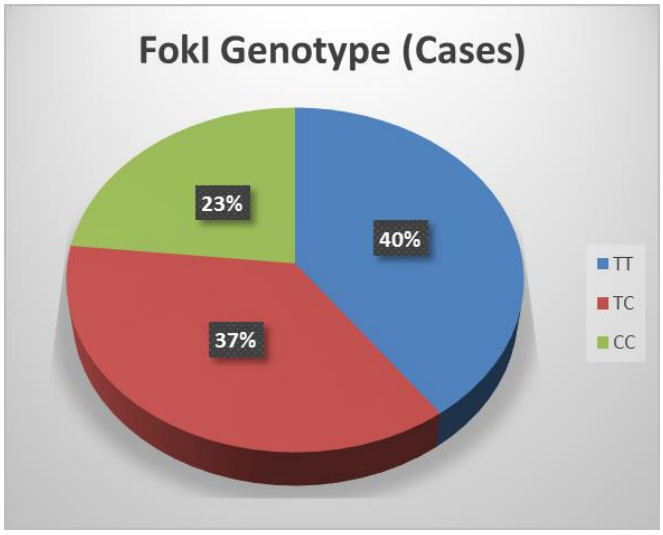


Table 7: Distribution of cases as per prevalence of FokI allele among cases

FokI Alleles	Cases	%
T	35	58.3%
C	25	41.7%
Total	60	100.0%

Among cases, prevalence of mutated T allele was 58.3% while that of wild C allele was 41.7%.

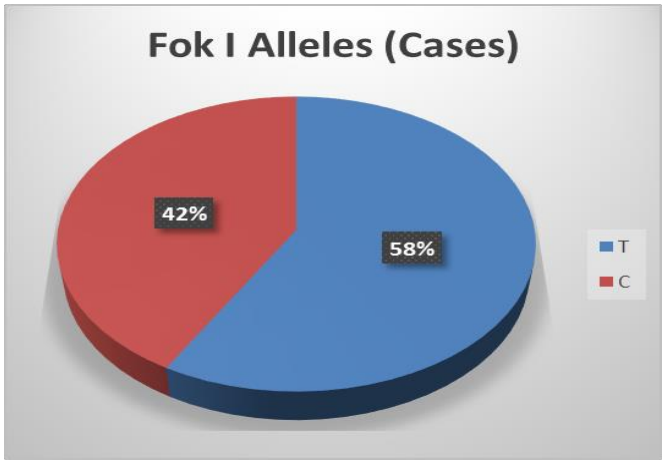


Table 8: Distribution of cases as per prevalence of FokI genotype among controls

FokI Genotype	Controls	%
TT	15	50.0%
TC	12	40.0%
CC	3	10.0%
Total	30	100.0%

Among controls, prevalence of mutated genotype (TT) was 50% while that of wild genotype (CC) as

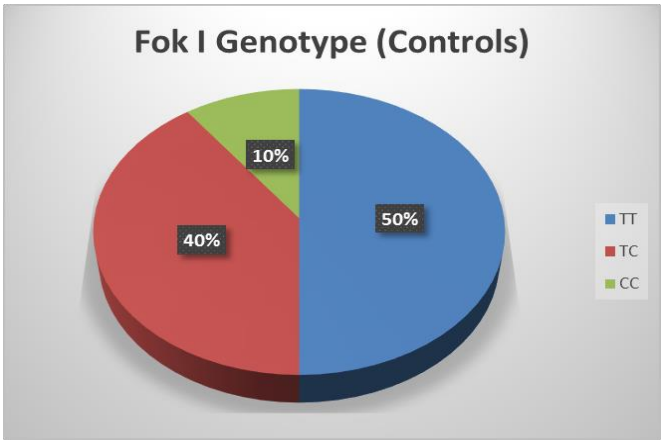


Table 9: Distribution of cases as per prevalence of FokI alleles among controls

FokI Alleles	Controls	%
T	42	70.0%
C	18	30.0%
Total	60	100.0%

Among controls, prevalence of mutated T allele was 70% while that of wild C allele was 30%.

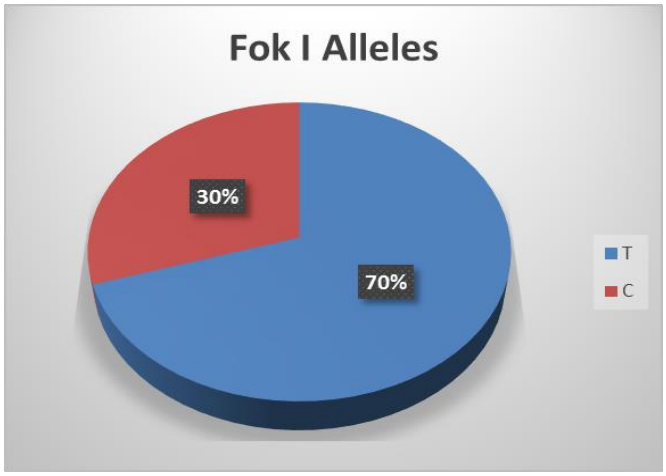


Table 10: Association of FokI genotype with lumbar disc degeneration

FokI Genotype	Group		Total
	Cases	Controls	
TT	12	15	27
	40.0%	50.0%	45.0%
TC	11	12	23
	36.7%	40.0%	38.3%
CC	7	3	10
	23.3%	10.0%	16.7%
Total	30	30	60
	100.0%	100.0%	100.0%
p- value - 0.37			

Prevalence of wild genotype (CC) was prevalent in 23.3% cases as compared to only 10% controls. However the difference was statistically non-significant (p-0.37).

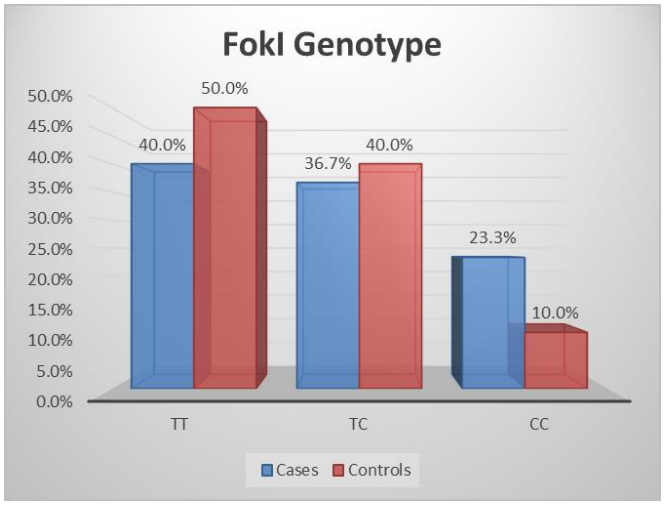


Table 11: Association of FokI allele with lumbar disc degeneration

FokI Alleles	Group		Total
	Cases	Controls	
T	35	42	77
	58.3%	70.0%	64.2%
C	25	18	43
	41.7%	30.0%	35.8%
Total	120	120	240
	200.0%	200.0%	200.0%
p- value - 0.25			

Prevalence of wild alllele (C) was prevalent in 41.7% cases as compared to 30% controls. The study shows that C allele was higher in cases with LDD, however the difference was statistically not significant.

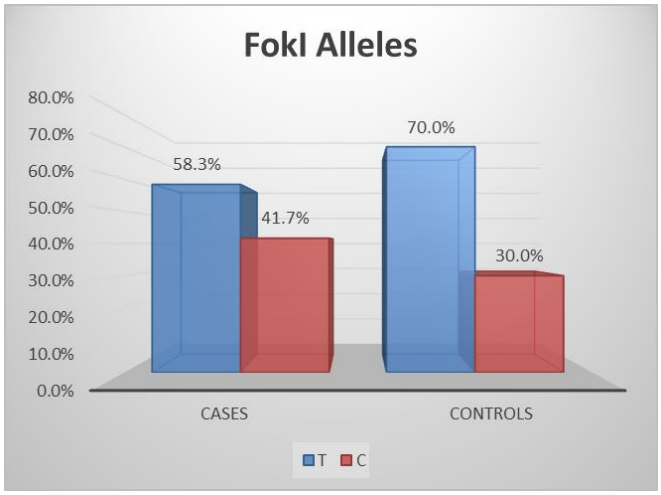
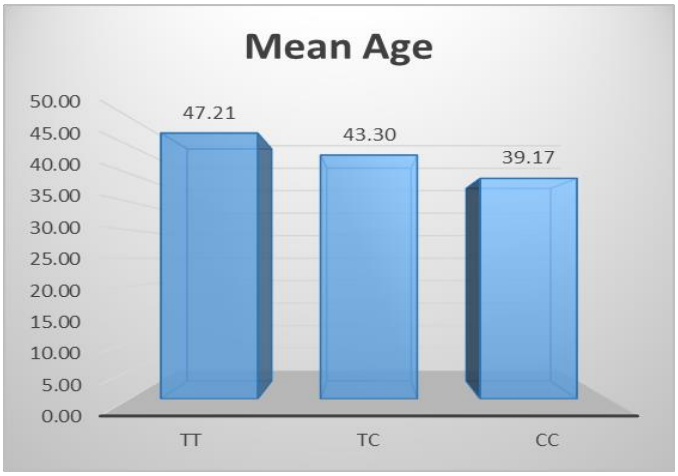


Table 12: Mean age at presentation among cases of lumbar disc degeneration

FokI Alleles	N	Mean	SD
TT	12	47.21	7.65
TC	11	43.30	7.86
CC	7	39.17	8.91
p- values <0.05			

Cases with CC genotype, developed LDD at a significantly younger age than cases with TT genotype (39.17 vs 47.21; p<0.05)



Discussion

In recent years, many gene polymorphisms have been reported to be associated with the occurrence of LDD. The most known and studied polymorphism sites have been identified in the VDR gene sequence including TaqI(rs731236), FokI (rs2228570) and ApaI(rs7975232).

It is generally recognized that VDR has an important influence on bony and cartilaginous metabolisms, including differentiation, proliferation, and maturation of cartilage cell. VDR is expressed in nucleus pulposus and annulus fibrosus cells of the vertebral disc, and has marked effect on proteoglycan synthesis. VDR gene variants have been supposed to be involved in the pathophysiology of the degenerated disc through the generation of an altered VDR expression.

The present hospital based case control study was thus conducted to demonstrate Vitamin D receptor (VDR) gene polymorphism (FokI) in subjects with lumbar disc degeneration (LDD) and to pursue the potential correlation between common allelic variants in vitamin D receptor locus and degeneration of the lumbar spine.

A total of 30 cases with varying degree of LDD were included in the study after proper informed consent in local language. A similar number of age and gender matched controls were also included.

Demography

Mean age of cases was 45.14 years with over half of them (56.7%) were between 31-50 years of age. A slight male preponderance was observed among study cases (56.7% males to 43.3% females).

Similar pattern was seem in other studies in which the incidence of low back pain was highest in the fourth decade, and overall prevalence increased with age until the 60-65 year age group and then gradually declines [12]. Persistent LBP is most common among people in their late thirties and early-to-mid forties [13].

The age pattern can be explained as degenerative changes is common in individuals above 40 years of age and its prevalence increases progressively to over 90% by 50 to 55 years of age [14]. Also, in young individuals (20 to 39 years) factors like repeated traumatic injuries and physical loading history can play a role in causing disk degeneration [15].

In a similar study by Savage RA to evaluate the relation between MRI, lumbar Spine and back pain; the mean age of study subjects was 47.13 with equitable gender distribution [12]. However Tsuji *et al*. and Takarad *et al*. reported slightly higher male prevalence in a similar studies.

Symptoms

Most common presenting symptom was found to be loss of lumbar lordosis (73.3%) followed by radiating back pain (63.3%), tingling/ numbness (33.3%) and loss of bowel/ bladder control (13.3%).

Radiation of pain is the significant finding in patients with LDD in many studies especially when it is associated with Sciatica [16]. In a study by RA Deyo *et al*. radiating back pain was observed in 70% of the patients with LDD [17].

The prevalence of tingling in patients with low back pain ranges from 5-47%. [132] In a study by Matthew ST *et al*. approximately 37.5% patients with LBP had tingling and numbness in atleast one region of one lower extremity [18].

Tsuji T. carried out a study to assess the relation between lumbar lordosis and LDD [13]. They studied 489 subjects between age of 50-85 years of age with complaints of back pain. There was a significant difference in lumbar lordosis in patients with and without back pain. Murrie *et al*. used magnetic resonance imaging (MRI) to assess lumbar lordosis in 27 patients with low back pain and 19 patients and 10 volunteers with no known back pain. The study was aimed to investigate whether lordosis is associated with low back pain. They concluded that loss of lumbar lordosis should be regarded as a strong clinical sign [19].

VDR Gene Polymorphism & LDD

In present study, we observed that prevalence of wild genotype (CC) was prevalent in 23.3% cases as compared to only 10% controls. Prevalence of wild allele (C) was prevalent in 41.7% cases as compared to 30% controls. The study shows that C allele was higher in cases with LDD, however the difference was statistically not significant. Cases with CC genotype, developed LDD at a significantly younger age than cases with TT genotype (39.17 vs 47.21; p<0.05).

The Finnish Twin Cohort study was the first genetic association investigation to report a statistically significant association between the VDR gene polymorphisms (TaqI and FokI) and risk of disc degeneration [20].

The association of the FokI polymorphism to LDD was subsequently confirmed in several population studies [21. 22] [22]. However, other studies were unable to replicate this initial finding.

Colambini *et al*. [21] in a similar study assessed 267 cases of LDD and 254 controls. CC genotype was a 2-fold risk factor to develop discopathies and/or osteochondrosis concomitant with disc herniation for both gender patients, while heterozygous CT was protective for females and TT genotype was protective for discopathies and/or osteochondrosis in males.

Vieira LA *et al*. [22] hypothesized a possible relationship between disc degeneration (DD) and VDR FokI/T2C polymorphism. The results disclosed statistical difference between allele distribution among cases and controls (p=0.025, odds ratio=1.58, confidence interval=1.07–2.32) considering VDR FokI/T2C polymorphism. The results

showed a positive association between VDR FokI/2C polymorphism and DD in Brazilian population.

Eskola P *et al*. [23] aimed to examine the associations between eleven putative predisposing single nucleotide polymorphisms (COL9A3, COL11A2, IL1A, IL1B, IL6 and VDR) and early disc degeneration (DD).They analysed the association between DD and single nucleotide polymorphisms revealed that the C-allele was more frequent among the subjects with DD, OR 6.71 [1.71-26.3].

In a systemic review by Pablam *et al*. [24], the analysis of various studies confirmed the protective role of FokI polymorphism, but suggested that its effect may be ethnic and gender specific.

Chen L *et al*. [25] also conducted a meta-analysis to investigate the association between VDR gene polymorphisms and intervertebral disc degeneration (IDD). Authors observed no association between VDR FokI polymorphism and IDD. However, on subgroup analysis by ethnicity, VDR FokI mutation was associated with a significantly lower risk for IDD among Caucasians.

Doraiswamy R *et al*. [26] conducted a study to validate the association of VDR polymorphisms and degenerative disc disease (DDD) in Indian population.Overall, the distributions of VDR genotype frequencies FF, Ff showed strong association with the LDD.

Thus from the observations made in present study and that by other authors, it can be deduced that mutant 'f'/ 'T' allele has somewhat protective role for lumbar disc degeneration. However further studies with larger sample size are required to validate this relationship in Indian population.

Conclusion

Degenerative disc disease was the commonest positive pathology observed in MRI lumbo-sacral evaluation of low back pain. Disc degeneration is a complex disease with an intricate interplay of multiple genetic polymorphisms. The present study was conducted on the hypothesis that there is a positive correlation between Vitamin D receptor (VDR) Fokl alleles frequencies distribution and lumbar disc degeneration (LDD).

The present study is the first of its kind towards understanding the genetic basis of degenerative disc disease in our set up. The Indian population forms one-sixth of the world's population and ethnically different from the other cohorts like Chinese, Caucasian in whom previous genetic studies have been done previously.

The study observed higher frequency of wild VDR genotype (CC) among cases of lumbar disc degeneration as compared to controls. Also, subjects with CC genotype, developed LDD at a significantly younger age. Though the difference was not significant statistically, which could be because of the relatively lower sample of the population, the present study provided useful information regarding common allelic variations in vitamin D receptor locus and degeneration of the lumbar spine. Present study thus conclude that VDR expression could be used as an important marker for monitoring lumbar disc degeneration in cases with lower back pain.

We recommend further studies with larger sample size to validate our findings. We also recommend further studies evaluating relation of other VDR gene alleles like TaqI, with degenerative disc diseases and also studies regarding association of different VDR gene alleles with specific types of degenerative disc diseases in males and females seperately.

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