Original Article

Degenerated intervertebral disc prolapse and its association of collagen I alpha 1 Spl gene polymorphism

A preliminary case control study of Indian population

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Abstract

Background: Degenerated disc disease (DDD) is a common disorder responsible for increased morbidity in a productive age group. Its etiology is multifactorial and genetic factors have been predominantly implicated. Disc prolapse results due to tear in the annulus, which is a fibrous structure composed largely of type I collagen. Functional polymorphism at the Sp1 site of the collagen I alpha 1 (COL1A1) gene has shown a positive association with DDD in Dutch and Greek populations. The purpose of this study was to assess COL1A1 Sp1 gene polymorphism in the Indian population.

Materials and Methods: Fifty clinically and radiologically proven patients with disc prolapse requiring surgery were included as cases and 50 healthy, age-matched volunteers served as controls. After isolating DNA from their blood sample, genotyping for COL1A1 polymorphism (rs1800012) was performed and identified as GG, GT, and TT.

Results: The mean age and body mass index in cases and controls were similar. 76% of the patients were males. The most common site of disc degeneration was L4–L5 (36%), followed by L5–S1 (34%). Homozygous–GG, heterozygous GT, and homozygous TT genotypes were seen in 38 (76%), 10 (20%) and 2 (4%) cases respectively, controls had similar percentage of genotypes as well. The alleles in cases and the control group showed no significant difference (P = 0.6744) and followed the Hardy–Weinberg Equilibrium in the study population.

Conclusion: The COL1A1 (rs1800012) is in Hardy–Weinberg equilibrium in the present subset of Indian population. But taken as a single factor, it was not found to be associated with DDD in this preliminary study. Disc degeneration is multifactorial and also anticipated to be a result of multiple genes involvement and gene-gene interaction.

Key words: Collagen I alpha 1 gene, degenerated disc disease, disc prolapse, gene polymorphism, Indian population
MeSH terms: Intervertebral disc, gene expression, gene proteins

Introduction

Low back pain is one of the most common symptoms of spinal abnormalities, with an annual point prevalence averaging 30%.[1] It has been recognized as one of the single largest cause for worker compensation and public health expenditure all over the world.[2]

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Intervertebral disc (IVD) degeneration is an aberrant, cell-mediated response to progressive structural failure.[3] Disc degeneration is a systemic phenomenon.[4] Commonly it affects lumbar followed by cervical and thoracic spine.[5] Etiology of disc degeneration is multifactorial in nature; it includes nutritional deficiency, mechanical load-bearing, injury/trauma and genetic factors.[6] Many researchers unanimously agree that genetic factors are largely responsible for the degeneration.[7-9] Disc degeneration is not only regulated by multiple genes and environmental factors,

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but also by gene-gene interactions and gene-environment interactions.[10] A recent paper using dynamic differential interaction network analysis identified potential biomarkers for degenerated disc disease (DDD).[11]

It is apparent from both candidate-gene approach and Genome-wide Association Studies that single nucleotide polymorphism plays an important role in increasing the risk of developing complex diseases like IVD.[12] Microarray and gene expression profiling implicate several extracellular matrix proteins in the etiology of DDD.[13]

Degeneration of the lumbar spine has been characterized with three stages: (i) Dysfunction phase (ii) instability phase and (iii) stabilization phase.[14] The initiating factor for disc degeneration is damage to the endplate which alters the mechanical environment and also affects the nutritional pathways.[15] Subsequently, the hydrostatic nucleus becomes smaller and decompressed and thus more compressive load-bearing is taken up by the annulus. As the disc degeneration progresses, the nucleus pulposus begins to bulge and the annulus gets torn [Figure 1]. If complete disruption of the annular fibers occurs, herniation of the nucleus pulposus can occur; however, if the annulus remains intact, then the nucleus pulposus may continue to degenerate, leading to a loss in the IVD space.[14] Scanning electron microscopy has also demonstrated structural failure of the annulus fibrosus, delamination and matrix cracking resulting in radial tears in annulus in degenerated discs.[16]

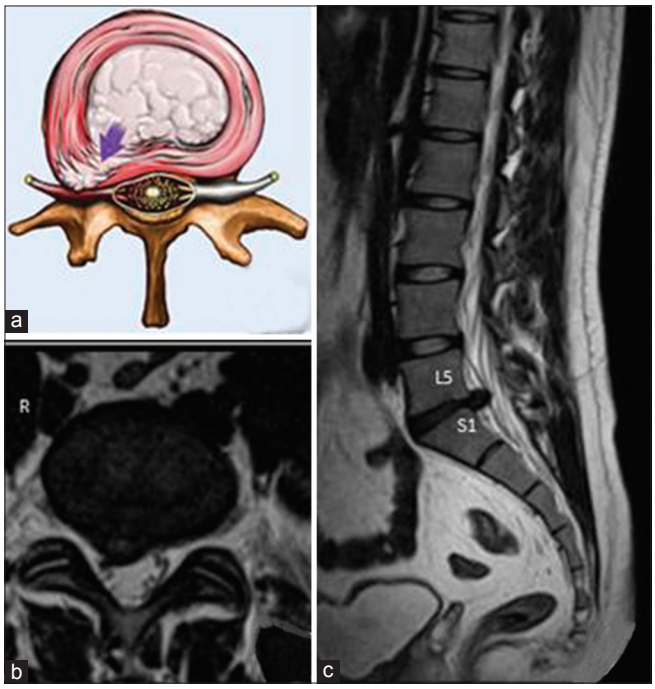

Figure 1: (a) pictorial representation showing disc prolapse due to tear in the annulus (Purple arrow) (b) magnetic resonance imaging (MRI) T2W axial cut and (c) MRI T2W sagittal images showing disc prolapse at L5S1 level

The collagen network of the disc is formed mostly by type I and type II collagen fibrils, which makeup approximately 70% and 20% of the dry weight of the annulus and nucleus, respectively.[17] The annulus is a fibrous structure composed largely of type I collagen. Disruption of the annulus in degenerated discs which results in disc herniation in explicit DDD patients can be explained due to the defective development of collagen because of some gene polymorphism.

Type I collagen consists of two alpha-1 and one alpha-2 chains, which are encoded by the collagen I alpha 1 (COL1A1) and COL1A2 genes, respectively. COL1A1 gene is located at the 17q21.3-q22 and is 18 kb (kilo bases) in size and is composed of 52 exons. In Sp1 polymorphism, the guanine (G) is substituted by thymidine (T) in the fourth Sp1 binding site in intron 1 of COL1A1, more specifically – in the promoter +1245 base pair (bp) from the transcription start site [Figure 2]. Functional polymorphism at this Sp1 site from G to T of the COL1A1 gene is thought to be associated with DDD.[18] The COL1A1 gene polymorphism has been studied in only Dutch[19] and Greek[20,21] population and has shown a positive association. Till date, no related studies have been reported in Indian population and to fulfill this lacuna, the present study was undertaken.

Materials and Methods

The present case control study was carried out at our institute in the Department of Neurosurgery in collaboration with the Department of Genetics and Molecular Medicine after obtaining ethical clearance from the Institutional Ethics Committee.

Selection of study group

Fifty patients with intervertebral degenerative disc prolapse and 50 age-matched controls that fulfill the inclusion

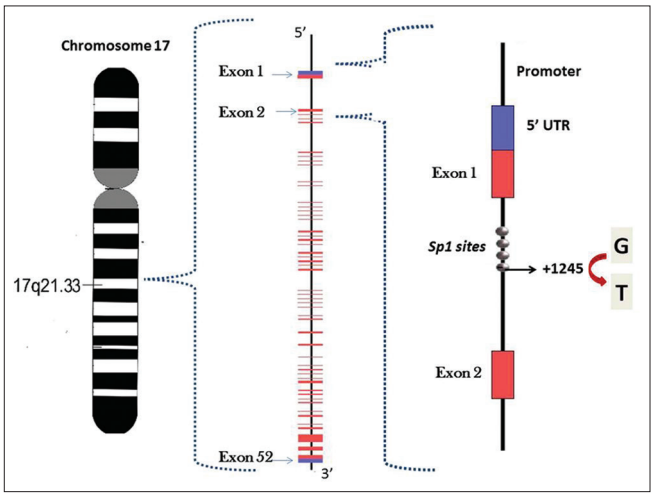

Figure 2: Diagrammatic representation of collagen I alpha 1 gene locus on chromosome 17, and functional polymorphism at the Sp1 site from guanine (G) to thymidine (T)

criteria were selected for this study which was carried out between Nov 2009 and Nov 2012. All subjects underwent standardized clinical evaluation. Magnetic resonance imaging (MRI) was obtained during symptomatic period with 1.5 Tesla machine. The Inclusion criteria of cases were: (i) Age group 18–60 years (ii) occupation not involving rigorous activities (iii) clinical evidence of disc disease with pain of more than 3 score (of 0–10 scale) on verbal rating scale (VRS) and visual analog scale (VAS) (iv) duration of pain along with or without radiculopathy and failed conservative management for a period of at least 3 months and (v) MRI lumbar spine sequences showing evidence of disc degeneration of grade 3 and 4 of Schneiderman's classification[22] and X-ray cervical spine lateral view with evidence of grade 3 or 4 of Kellgren classification[23] (vi) MRI sequences with evidence of disc prolapse/extrusion/sequestration. The exclusion criteria were: (i) Individuals above 60 years of age (ii) occupations like manual laborers lifting heavy weights or persons dealing with vibratory tools (iii) body mass index (BMI) more than 30 and (iv) smokers. Fifty healthy, age matched volunteers, without any history of neck or back pain and medical or surgical history for disc prolapse/degeneration served as controls.

DNA isolation

Written consent was obtained from all cases and controls and 2 mL venous blood sample was collected in a sterile vacutainer with ethylene diamine tetra acetic, which was used for molecular analysis. Genomic DNA was isolated by the method routinely used in our Genetic Laboratory as described by Vattam *et al.*,[24] DNA was stored at –20°C until processed. Genotyping for the COL1A1 polymorphism was performed by polymerase chain reaction using specific primers synthesized from Bioserve Biotechnology Ltd., (Hyderabad, India) which were also used by Grant *et al*.[25] and Mann *et al*.[26] Forward primer (5' → 3'): 5' TAACTTCTGGACTATTTGCGGACTTT TTGG 3'. Reverse primer (3' →5'): 3' GGAGGTCCAGCCCTCATCCCGCCC 5'.

PCR was carried out using Taq DNA polymerase using 96 well thermal cycler. DNA was initially denatured, followed by annealing and then extension. The amplified products were digested and electrophoresed on 2% Ethidium bromide agarose gel for identifying the amplification product. Gels were imaged and analyzed in ultra violet light as per the standard protocol.[27,28]

Statistical analysis

Continuous data are reported as mean ± standard deviation and categorical data as the number (percentage).

Results

The demographic characteristics of DDD patients and healthy controls are depicted in Table 1. Equal number (n = 50) of patients and controls were included in this study as per protocol and most of them, (40 cases and 45 controls) were of younger age (age < 50 years) in both the groups (P = 0.1688). The mean age and BMI in both the groups were similar, but males were significantly more in the DDD group than the controls. Three patients (6%) gave positive family history of at least one first-degree relative with DDD, who had also undergone surgery, whereas none of the controls had such positive family history (P = 0.1882). Genetic analysis was carried out in all the 100 samples as per our institutional genetic analysis protocol (described before). GG genotype is indicated by a single band at 233 bp, GT by two bands at 233 bp and 264 bp and TT by one band at 264 bp on gel electrophoresis picture [Figure 3].

The homozygous GG, heterozygous GT and abnormal homozygous TT were seen in 38 (76%), 10 (20%) and 2 (4%) of DDD patients and in 39 (78%), 10 (20%) and 1 (2%) of healthy controls, respectively. [Figure 4] Allele frequencies were estimated by the gene counting method and Chi-square test was used to identify departures from Hardy–Weinberg equilibrium. It was found that this

Table. 1: Demography of the cases and controls

Characteristics	Cases (n=50)	Controls (n=50)	P
Mean age (mean±SD) (in years)	41.7±11.9	43.6±6.7	0.3276
Gender - number of males (%)	38 (76)	16 (32)	<0.0001
BMI (mean±SD)	25.67±1.64	25.22±1.36	0.1385
Positive family history (%)	3 (6)	0	0.1882

SD=Standard deviation, BMI=Body mass index

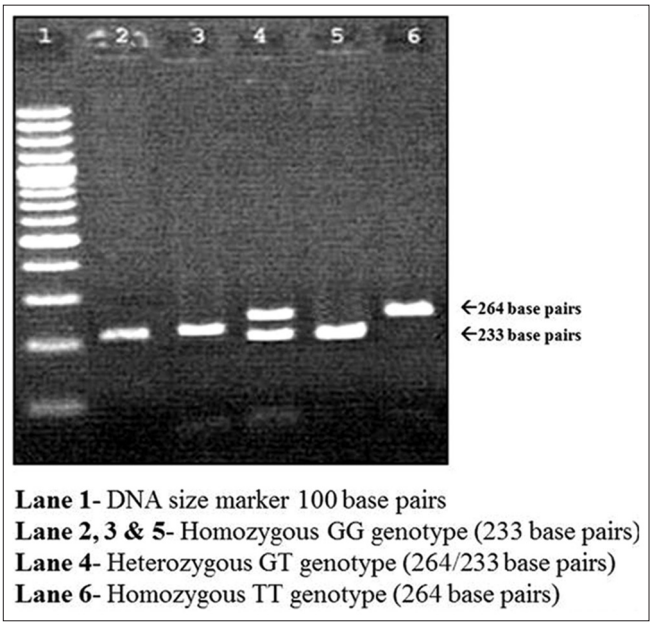

Figure 3: Ethidium bromide-stained 2% agarose gel picture showing bands corresponding to GG, GT and TT genotypes

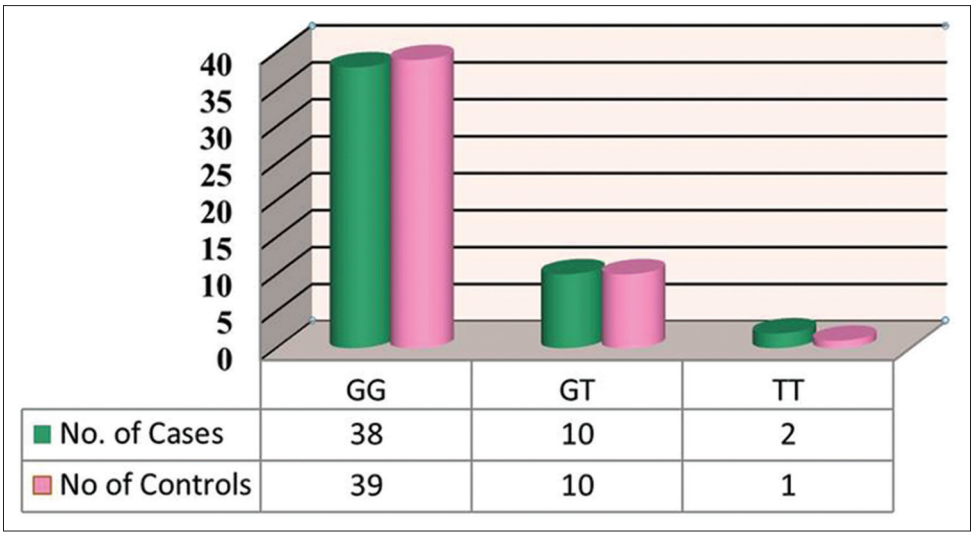

Figure 4: Collagen I alpha 1 gene polymorphism in cases with intervertebral disc disease and healthy volunteers

Table 2: Expected frequency of different genotypes based on Hardy–Weinberg equilibrium in cases and controls

Genotype	Cases			Controls		
	Expected	Observed	Hardy-Weinberg equilibrium %	Expected	Observed	Hardy-Weinberg equilibrium %
Common homozygotes GG	36.98	38	73.96	38.72	39	77.44
Heterozygotes GT	12.04	10	24.08	10.56	10	21.12
Rare homozygotes TT	0.98	2	1.96	0.72	1	1.44

χ^2=1.44 (*P*=0.2309)

polymorphism follows the Hardy–Weinberg equilibrium in the study population [Table 2].

The risk of disc degeneration for the people with TT genotype does not show a significant difference with those of GG genotype in the population ($P = 0.5639$). Also when the genotypes were matched for dominant, co-dominant, and recessive models statistically, significant difference was not observed [Table 3]. It was observed that odds ratio of G allele and T allele as compared in cases and control groups showed no significant difference ($P = 0.6744$). Thus, we can conclude that T allele (abnormal variant gene) was not responsible for degenerative disc disease in our population.

In our series, eight cases (16%) had cervical disc prolapse whereas 84% had lumbar disc prolapse. The most common site of disc degeneration was L4–L5 (36%), followed by L5–S1 (34%). COL1A1 genotypes were evaluated with site of disc degeneration (i.e., cervical and lumbar region) to ascertain their association [Table 4]. In this population, the heterozygous GT genotype was present in more number of patients with disc degeneration at the lumbar region as compared with cervical level ($P = 0.0009$).

All the cases underwent surgery and were analyzed for symptoms and neurological assessment at 7th postoperative day and after 6 weeks of surgery. VAS and VRS for pain showed significant relief from the pain in all cases at 7th postoperative day and after 6 weeks of surgery as compared to preoperative score ($P < 0.0001$). None of the patient had any new neurological deficit, symptoms or bowel-bladder dysfunction after surgery; however, sensory and motor deficit was persistent in 54% of cases until 6 weeks of followup. The genotype of these DDD patient with neurological deficit, when matched with genotypes of those without neurological deficit did not show a significant difference (TT vs. GG, $P = 0.3829$). Furthermore, the odds ratio of G allele and T allele when compared in the above subgroups showed no statistical difference ($P = 0.1360$).

Table 3: Association of gene polymorphism

Genotypes groups compared	OR	95% CI	*P*
TT+GT versus GG (dominant model)	1.1196	0.3175-1.8124	0.5341
TT versus GT+GG (recessive model)	2.0417	0.1792-23.2672	0.5653
GT versus GG+TT (co-dominant model)	1.0000	0.3753-2.6645	1
T allele versus G allele	1.5315	0.3753-6.2502	0.5525

OR=Odds ratio, CI=Confidence interval

Discussion

Intervertebral disc degeneration is a complex, multifactorial disorder where both environmental and genetic factors play a role and are responsible for the increased prevalence of morbidity. Genome Wide Association studies and Candidate

Table 4: Frequency of genotypes and alleles in cases with disc degeneration at cervical and lumbar region

Genotype/Allele	GG genotype (%)	GT genotype (%)	TT genotype (%)	G allele (%)	T allele (%)
Cervical (*n*=8)	6 (75)	1 (12.5)	1 (12.5)	13/16 (81.25)	3/16 (18.75)
Lumbar (*n*=42)	32 (76.19)	9* (21.42)	1 (2.38)	73/84 (86.75)	11/84 (13.09)
Controls (*n*=50)	39 (78)	10 (20)	1 (2)	88/100 (88)	12/100 (12)

*χ^2 for GT genotype at lumbar versus cervical level, *P*=0.0009; and lumbar level versus controls, *P*=0.8981

Gene Polymorphisms analysis have shown that more than 20 genes may be involved in the etiology of DDD.[29]

Collagen I alpha 1 is a major component of the fibrous structure of annulus in IVDs and has been studied by several authors in osteoporosis[25,30,31] and DDD.[19-21,29,32] Grant *et al*.,[25] described a novel G→T polymorphism in a regulatory region of COLIA1 at a recognition site for the transcription factor Sp1 that was significantly related to bone mass and osteoporotic fracture in two population of British women. Subsequently Pluijm *et al*., showed a three-fold significant association of COL1A1 gene with degenerative disc disease patients from Amsterdam,[19] and Tilkeridis *et al*., in 36 Greek young military recruits.[20] In the small sample size, they found a significant association of TT genotype with disc degeneration ($P = 0.001$). Latter Bei *et al*., reinforced the association of this polymorphism in same Greek population.[21]

Ninety-nine polymorphisms in 29 selected candidate genes were evaluated and COL1A1, COL9A1 and COLL11A2 were shown to be associated with disc signal intensity.[32] In a recent study, it was found that collagen type IX and not the collagen I was associated with disc degeneration by Mayer *et al*.[29] A similar association between collagen IX polymorphism and disc degeneration was reported in Indian patients earlier by Rathod *et al*.[33] However there are no studies to the best of our knowledge assessing COL1A1 polymorphism in Indian patients with IVD degeneration.

The present preliminary study showed that COL1A1 Sp1 polymorphism "rs1800012" was present in Indian population and follows the Hardy–Weinberg equilibrium but does not appear to be associated with Disc degeneration in our population. The T allele of this gene was however seen more in lumbar and cervical disc patients as compared to controls, but the difference was not statistically significant. The GT genotype was significantly higher ($P = 0.0009$) in lumbar disc disease compared to cervical disc disease, however, the sample size is small. This particular polymorphism also did not show any correlation with surgical outcome. The results of the present study indicate that either another polymorphism of collagen I or a polymorphism of another collagen type may play a more direct role in IVD degeneration in the Indian population.

There are some limitations of our study. Since most of the patient included in the study were from Southern part of India, it is possible that patients from other parts of India may show an association similar to that observed in Dutch and Greek populations.[19-21] Second, we have included Schneiderman classification to assess the degeneration at the lumbar region, which has been criticized for poor intra and interobserver reliability. Third, it is impossible to predict that the age-related healthy individuals who served as controls will never have disc degeneration and herniation at a later date.

This is the first study which evaluated COL1A1 "rs1800012" polymorphism and showed results similar to Mayer *et al*.,[29] in the American Caucasian population. A larger study with more Candidate Gene polymorphism is required to identify biomarkers associated with IVD degeneration in the Indian population.

Conclusion

Intervertebral disc degeneration is a complex disease and several genes are associated with its etiology. The present preliminary study has looked at the role of a possible candidate gene polymorphism, Sp1 site of COL1A1. This rs1800012 is in Hardy–Weinberg equilibrium in the subset of Indian population studied but was not found to be associated with degenerative disc prolapse.

There is a need to study this polymorphism in a larger population to have confirmatory result as well as to evaluate other probable candidate genes like Vitamin D receptor, aggrecan, type IX collagen, asporin, MMP3, interleukin -1 (IL-1), and IL-6 to eventually develop a predictive model for identifying individuals at high risk of disc degeneration. Such genetic studies are also crucial for understanding the molecular mechanism of the IVD degeneration.

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Conflicts of interest

There are no conflicts of interest.

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