GENETIC AND ENVIRONMENTAL FACTORS INVOLVED ON INTERVERTEBRAL DISC DEGENERATION

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SUMMARY

The etiology of intervertebral disc degeneration (IDD) has not been fully clarified yet. Vitamin D receptor's gene (VDR) has been suggested as one of the potential entities involved in disc pathologies onset. On the other hand, this study correlates, for the first time, glutathione transferases M1 and T1 genes (GSTT1 and GSTM1) participation, which are responsible for cigarette components' inactivation, in IDD. DNA was extracted from leukocytes of 66 patients and 88 controls, paired by gender and age. The VDR-FokI polymorphism was amplified by polymerase chain reaction (PCR) followed by restriction with FokI enzyme. GSTT1/M1 polymorphisms were determined by means of PCR multiplex. Family history and disease severity were highlighted in patients carrying the f allele of the VDR-FokI gene (P=0.000 and 0.0012, respectively). The age at disease onset has shown to be early in individuals with _/f genotype (average 26 years old). A correlation was found between FokI polymorphism and early degeneration and IDD severity, with smoking habit also interfering in this process, regardless of the presence or absence of a favorable genotype for GSTT1/M1.

Keywords: *Risk factors; Polymorphism, genetic; Intervertebral disc.*

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INTRODUCTION

The intervertebral disc loses its hygroscopic power with age, causing a progressive dehydration process, characterizing a discopathy. From intervertebral disc degeneration, the spine begins to show a progressive instability on the involved area, leading to a number of further events on the other elements of the segmental functional unit. This may occur on any region of the spine; however, it is more frequently found between the fourth and fifth lumbar vertebrae, and between the fifth lumbar and first sacral, at the age of thirty – forty years approximately[1].

The precise etiology of IDD has not been fully explained. Until recently, it was attributed uniquely to the accumulation of environmental effects, primarily micro or macro, trauma, lifestyle, smoking habit, atherosclerosis, added by changes occurring on discs structure with age. Nevertheless, more recent researches show a moderate influence of these factors on disc degeneration, which reinforces the relevance of a genetic-related factor for this disease[2,3]. Despite of that, the natural evolution of the degeneration – which is genetically determined – may be modified to a certain extent by changing lifestyles and the environment[4].

I. Polymorphism of Vitamin-D receptor gene on disc degeneration

The active form of Vitamin-D, the 1α,25 di-hydroxy-vitamin D [1α,25-(OH)2D3] or Vitamin-D3, is necessary for cell growth and differentiation on many tissues. Furthermore, it is involved on bone mineralization process, calcium absorption on the intestine, phosphorus homeostasis, and parathyroidal hormone regulation. Additionally, it participates on the differentiation, proliferation and maturation of cartilaginous cells, also influencing proteoglycans synthesis through joint chondrocytes[5].

The vitamin-D receptor (VDR) is a member of the super family of hormonal nuclear receptors regulating the transcription. Some of these members must be highlighted, including: the steroid and retinoic acid receptors (RXR), which have a steroid-bonding domain, an interaction domain with receptors' 'super family' members, and a third DNA-bonding domain, which contain two zing fingers reasons, located on protein's N-end portion[6].

Vitamin D3 [1α,25-(OH)2D3] is liposoluble and, when entering into the cell, it promptly interacts with its receptor (VDR). After the VDR is activated by getting bonded to Vitamin D3, this receptor may, either separately or as a heterodimer with several other super family's receptors, particularly RXR, be bonded to the specific regulation sequence, present on the promoters of genes responsive to vitamin D. Following the heterodimer formation, transcriptional factor IIB (TFIIB) and its co-factors are recruited, thus starting transcription[7].

The FokI polymorphism of the VDR gene results from a change on transcription initial site, located at exon 2. That site, highly preserved in mice, rats and human beings, suggests that the polymorphism on VDR's exon 2 could affect VDR protein functioning[8].

II. Environmental Factors on Intervertebral Disc Degeneration

Smoking habit induces biochemical stress to several tissues. Such stress is part of the environmental components contributing to nutrition and blood flow changes on the disc, promoting a discogenic disease. There also are two additional kinds of stress, the dynamic and the physiological one. The dynamic stress involves heavy activities, overweight, posture errors, and accidental injuries, while the physiological one is associated to the aging process[9].

Among the most toxic chemical components of tobacco, nicotine, benzpyrene, and other polycyclic aromatic hydrocarbons are found. The latter lead to endothelial injuries, while carbon monoxide, resulting from combustion, reduces red blood cells' ability to transport oxygen. Catecholamine increase also occurs, resulting in vasoconstriction, further limiting oxygen supply to tissues and causing damages to vertebrae and bones. There are epidemiological evidences showing that chronic smokers suffer from early degeneration of the intervertebral disc, having less resilient vertebrae, in addition of presenting bone demineralization advancement, which may hasten osteoporosis development[10].

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Chemical compounds present in cigarettes are biometabolized, being oxidized on phase I, mainly, by the enzymes belonging to cytochrome P-450 (CYPs)' super family, and inactivated on phase II by the conjugation of the functional group of products formed with a hydro soluble endogenous byproduct (glutathione, sulfate, glucose, acetate) by means of the glutathione-S-transferases (GSTs), UDP-glucoronyltransferases and N-acetyltransferases (NATs). Thus, metabolites are transformed into hydrophilic compounds, more easily excreted[11].

Individual differences on the ability to respond to genotoxic and carcinogenic compounds are bonded to the presence of polymorphisms on enzymes involved on the metabolism of chemical compounds, with this inter-individual variability playing a major role in diseases development.

Among phase-II enzymes, GSTs play a major role on detoxification processes. The gene *GSTT1*, located on human's chromosome 22, is polymorphic. Its homozygous deletion may occur, resulting in a null genotype *(GSTT1 0/0)*, of which frequency depends on ethnical origin. The gene codifying to isoform *GSTM1* is located at chromosome 1, is polymorphic, presenting four allelic variants, the *GSTM1*A*, *B, *C, and *0. The first two are not different regarding the kind of byproduct, while the allele *C is extremely rare and the variant *0, which is the null allele, causes absence of enzymatic activity when in homozygosis[12]. Thus, the presence of at least one functional allele is enough for the enzyme to function by playing its role.

In brief, the smoking habit seems to be involved in intervertebral disc degeneration as an important risk factor for this disease in human beings.

OBJECTIVES

The purposes of this study include the following:

1. Identify polymorphisms on the vitamin-D receptor gene (VDR) and the codifying gene for glutathione S-transferases M1 and T1 (GSTM1 and GSTT1) in a group of patients with intervertebral disc degeneration, as well as in a control group;
2. Associate the polymorphisms described above with the findings obtained from nuclear magnetic resonance, clinical data, familial history and age at the onset of the disease, as well as anthropometric (overweight) and environmental (smoking habit) factors;
3. Correlate anthropometric, occupational, environmental and familial history factors for discogenic disease between patients and controls.

MATERIALS AND METHODS

I. Case series

Sixty six patients were studied (38 males and 28 females), not related and screened in a spine-specialized clinic in São Paulo city. All patients were Caucasians, ages ranging from 16 to 62 years (average: 38 years old), presenting with clinical and Magnetic Resonance (MR) imaging diagnosis of discopathies, with or without associated disc hernias. Individuals with disc changes proven to be resultant from previous trauma, history of jobs requiring carrying heavy loads, excessive efforts, use of vibrating machines, or performing impact activities were excluded from the study. The patients were paired to 88 Caucasian subjects, ages ranging from 16 to 57 years (average: 41 years old), being 35 males and 53 females, screened at the Medical College in São José do Rio Preto (FAMERP) and at Paulista State University "Júlio de Mesquita Filho", campus São José do Rio Preto, clinically assessed and diagnosed as discopathies-free. All subjects enrolled have been informed about the objectives of the study, after which they signed an Informed Consent Form. The study in reference was approved by the Ethics Committee of the Paulista State University (Unesp).

II. METHOD

The enrolled subjects were submitted to peripheral blood collection and answered to a detailed questionnaire about sports and occupational activities, kind of diet, habits, and provided their anthropometric measurements for calculating the body mass index (BMI) [weight (Kg)/ height ^{2} (m)].

II. 1. Patients' Assessment

Patients' assessment was performed by an expert doctor in spinal diseases, with diagnosis being corroborated by another qualified investigator. The analysis consisted of anamnesis, familial history for spinal diseases, physical examination, and MR imaging studies of the spine. From the MR, some data were extracted such as the presence of discopathy and herniation at different degrees (convexity, protusion, extrusion), sign intensity, height, fibrous ring and pulpous nucleus rupture, arthritic injuries and ligament degradation. Herniation is understood as the intervertebral disc protruding out of its space to the vertebral body; sign intensity degree as a change on disc color, ranging from light gray to black, with darkening being associated to a dehydration or degeneration process; height as the degenerative process parameter, with its reduction being associated to picture evolution; fibrous ring and pulpous nucleus rupture as a continuity gap on its tissues, and; arthritic injuries as joints degeneration. Figures 1 and 2 show degeneration signs assessed on patients.

The disease severity degree was identified according to the modified Scheneider's classification [5]. In this case, Degree 1 was characterized by the presence of 1-3 signs above (no patient showed less than 2 signs); Degree 2, with 4-5 signs, and Degree 3 showing 6-7 signs of disc degeneration. Similarly, severity levels were attributed to the disease, with levels 1 and 2, respectively, employed in cases of injury present in one or more spinal segments.

II. 2. Genotyping

In each subject, 5mL of peripheral blood were collected. The DNA was obtained by using the extraction technique as described by Gustincich *et al*.[13].

The amplification of the polymorphic segment of the VDR-*FokI* gene occurred by polymerase chain reaction (PCR). The detection of the *FokI* polymorphism followed the protocol described by Harris et al.[14]. The *primers*: 5'-AGCTGGCCC TGGCACTGACTCTGCTCT-3' and 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3' were diluted into 1.5 mM magnesium chloride, 60 mM Tris HCl, pH 9.0, 15 mM NH_4SO_4 10% dymethylsulfoxide (DMSO), dNTPs (200[um]M each), 0.25μL Taq polymerase and genomic DNA at 200ng/50μL strength. The amplification conditions were as follows: 94^{o}C for 30 seconds for DNA denaturation, ringing temperature of 60^{o}C for 30 seconds, 72^{o}C for 30 seconds during 35 extension cycles. Generated byproducts were digested by *FokI* enzyme at 37^{o}C for 3 hours, and then submitted to electrophoresis in 2% agarose gel, containing Tris-EDTA tampon and ethidium bromide (10mg/mL). The homozygous genotype named FF, does not contain the restriction site for FokI enzyme, thus resulting in a full segment of 265 pb. The presence of the restriction site for this enzyme results in a fragment of 196 pb and a 69 pb else. In these conditions, the homozygous genotype is categorized as ff.

Thus, the heterozygous genotype Ff contain three bands, one of 265 pb, other of 196pb, and the last one of 69 pb.

The polymorphisms of the genes *GSTT1* and *GSTM1* were detected by genic amplification by means of polymerase chain reaction (PCR Multiplex) described by Abdel-Rahman et al.[15]. For PCR procedure, 30 pmol of each of the *primers* for genes *GSTM1* (5'-GAACTCCCT-GAAAAGCTAAAGC; 5'-GTT-GGGCTCAAATATACGGTGG), *GSTT1* (5'-TTCCTTACTGGTCCT-CACATCTC; 5'-TCACCGGAT-CATGGCCAGCA) and *CYP1A1*

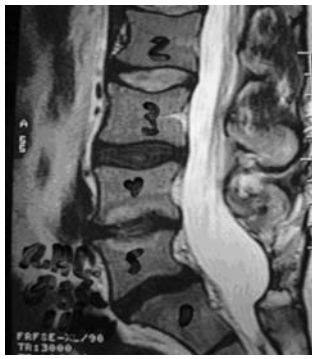

***Figure 1** - Nuclear magnetic resonance image, at sagittal plane, showing discopathies at L3-L4, L4-L5 e L5-S1 levels (black discs).*

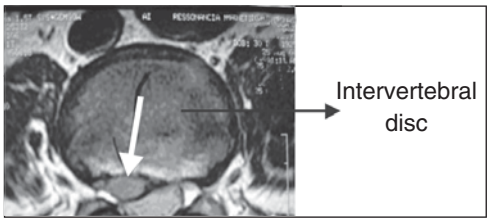

Figure 2 - *Nuclear magnetic resonance image, at axial plane, showing disc hernia (white arrow).*

(5'-GAACTGC-CACTTCAGCT-GTCT; 5'-AGCT-G C A T T T G - GAAGTGCTC) were used, with the latter being used as an amplification internal control. The reaction mixture was achieved with 1.5 mM magnesium chloride, 60 mM Tris HCl, pH 9.0, 15 mM NH_4SO_4, dNTPs (200[um]M each), 0,25μL Taq polymerase and genomic DNA at 200ng/50μL strength. The matter was processed in an automatic thermocycler, being initially submitted to a temperature of 94°C for 4 minutes for DNA pre-denaturation and then for 40 cycles with denaturation settings at 94°C for 2 minutes, *primers* ringing at 59°C for 1 minute and chain extension at 72°C for 1 minute. The final extension occurred at 72°C for 10 minutes.
PCR byproducts were submitted to electrophoresis in 2% agarose gel, stained with ethidium bromide (10mg/mL). The reaction byproducts were seen as fragments of 480 pb for gene *GSTT1* presence, 215 pb for gene *GSTM1* presence, and 312 pb for gene *CYP1A1*. In the absence of *GSTT1* and *GSTM1*, meaning the null genotype (0/0), only the intermediate band corresponding to gene *CYP1A1* was seen (mandatory presence in all individuals, since a mutation of that gene would be deleterious).

II. 3. Statistical analysis

The logistic regression analysis was employed for examining the correlation between smoking characteristics, BMI, familial history and polymorphisms of the genes *VDR*, *GSTT1* and *M1*, being also compared by variance analysis. The correlation of the genotypes with the different disc degeneration degrees (1, 2 or 3) was assessed by Mann-Whitney's test. The differences between studied polymorphisms frequency and the injury levels of the disease (1 or 2 levels) were calculated by Fisher's exact test. The t-test was employed for correlating between mean age at the onset of the disease with genotypes and other risk factors. For a comparison between disease levels and severity degrees, genotypes and lifestyles, the Fisher's or the Chi-Squared tests were used. The end results were comprised in a confidence interval of 95% and the P value below 0.05 was considered as statistically significant.

RESULTS

I. Allelic and Genotypic Frequencies for *VDR, GSTT1* and *GSTM1*

Table 1 shows the distribution of allelic and genotypic frequencies for VDR gene in patients with discogenic disease and their corresponding controls. The f allele frequency was significantly higher in patients (0.46;P=0.0001). The FF genotype was higher in control subjects (69.3%) as compared to patients (13.6%; P=0.0001), while the Ff genotype was shown to be significantly more frequent in patients (81.8%; P=0.0001). No differences were found in genotypic frequencies for *GSTT1* and *GSTM1* between patients and control subjects regarding the presence (+/+) and absence (0/0) of the gene (P=0.84 and P=0.14, respectively).

II. Personal history

Lumbar pain or disc hernia was mentioned as familial history, preferably by patients as compared to control group (78.8% ; 34.1%, respectively, P< 0.001), according to Table 2, which also shows the other characteristics, such as smoking habit and overweight, similar between groups.

III. Correlation between Personal History and *VDR*, *GSTT1* and *GSTM1* Polymorphisms

Table 3 shows the correlation between VDR-*FokI* polymorphism with tobacco use, overweight and familial history in patients and control subjects. Frequency, which has been assessed in conjunction of at least one allele f (_/f) with positive familial history, has shown to be significantly increased among patients (90%) when compared to control subjects (0%; P<0.001). On the other hand, the higher frequency of FF genotype prevailed in smoker patients (80%) when compared to control subjects (40%; P<0.001).
Regarding the age at disease onset, we found a significantly higher average age in patients with the FF genotype (38 years, ± 11.9) when compared to patients with genotype _/f (26 years, ± 10.2; P= 0.013). When this characteristic was associated to familial history, the mean age at disease onset for individuals with the FF allele was 33 years (± 10.7), thus higher than those with the -/f allele, who presented a mean age of 26 years (± 10.5). Similarly, the mean age at disease onset in individuals with no familial history carrying the FF allele was 41 years, ± 13.3, higher than -/f individuals (28 years, ± 7.8) (Table 4). No significant differences were found between genotypes and personal history in patients and control subjects.

IV. Clinical Data and Genotyping for VDR-*FokI*, *GSTT1* and *GSTM1*

Table 5 shows the correlation of VDR-*FokI* genotypes of patients with the severity degree and injury level, as assessed by magnetic resonance. Genotypes with at least one allele f (_/f) showed a correlation with the increasing severity degree for disc degeneration (28.8%) when compared to FF genotype (0.0%; P=0.0012). The same did not happen to injury levels.
There was no correlation between absence of at least one of the *GSTT1* and *GSTM1* genes and increased severity or spinal injury levels.

DISCUSSION

The results reported in this study express the existence of genetic factors on the susceptibility to intervertebral disc degeneration. The

Allele	Patient		Control		P*
	N	frequency	N	frequency	
F	72	0.54	149	0.85	
F	60	0.46	27	0.15	0.0001
Total		1.00		1.00	
Genotype	**N**	**%**	**N**	**%**	
FF	09	13.6	61	69.3	0.0001
Ff	54	81.8	27	30.7	0.0001
Ff	03	4.6	0	0	0.0767
Total	66	100	88	100	

*Fisher's exact test; N= number of subjects.

Table 1 –*Distribution of allelic and genotypic frequencies for VDR-FokI polymorphism in patients with disc degeneration and in control subjects.*

Personal History	Patient		Control		P*
	N	Frequency %	N	Frequency %	
Smoking habit	20	30.3	15	17.0	0.055
Overweight**	10	15.5	21	23.9	0.224
Familial history	52	78.8	30	34.1	<0.001

*Test = logistic regression; ** Overweight: BMI=25 - 30kg/m².

Table 2 - *Distribution of personal history of patients with disc degeneration and of control subjects.*

allelic and genotypic distribution for *FokI* polymorphism of the vitamin-D receptor gene differentiates patients from control subjects, since the significantly increased frequency of allele f in patients with disc degeneration (0.46) was outstanding as compared to control subjects (0.15). The Ff genotype was present in most of the subjects affected by the disease (81.8%), while in control group there was a prevalence of the dominant FF homozygote (69.3%).

The frequency of genotypes for VDR-*FokI* in these populations varies between the different ethnical groups, with FF frequencies ranging from 28% to 35%, Ff from 48% to 50% and ff from 14 to 17% among white or Caucasian individuals aged between 20 and 62 years [3,14,16]. On the other hand, among black or African population, the FF genotype is prevalent (66%), followed by Ff (31%) and ff (4%)[14]. The numbers for control group in this study were 69.3%; 30.7% and 0.0%, respectively, with the low frequency found for allele f potentially being explained as a result of a small case series, added by the large and real miscegenation of the Brazilian population.

Videman et al.[3] point to a straight correlation between an increased IDD degree and the presence of each allele f. On this ground, individuals carrying the ff genotype showed to be most affected, decreasingly followed by Ff and FF genotypes. Consistently, in this study, the allele f has shown to be correlated to a higher degree of degeneration when compared to allele F (P=0.0012).

The onset of degenerative process in young patients strongly suggests the influence of genetic components [4]. Such correlation results from the fact that, in those individuals, the intervertebral disc has been exposed to environmental risk factors for a shorter period when compared to older individuals [5]. Our data corroborate this correlation, since the age at disease onset was lower among patients carrying the Ff or ff genotype (average: 26 ± 10.2 years), considering the VDR-*FokI* polymorphism, while the average for patients with the FF genotype was 38 ± 11.9 years. Interestingly, besides the early involvement of patients carrying the allele f, discogenic disease history in patients' families presented a similar profile. When this characteristic was correlated to patients' familial history and also correlated to the presence of the allele f, the mean age at disease onset was lower (26 ± 10.5 years) in patients with positive familial history, while the average for individuals with no familial history of disc degeneration was 33 ± 11.7 years (P= 0.025).

Therefore, the presence of discogenic disease in the family was found in the majority of the patients in the study group (78.8%), as opposite to control subjects, where this frequency has shown to be reduced (34.9%). From the total number of patients with positive familial history, 27% had the allele f, while none of the individuals in control group presented with this allele, even with positive familial history for the disease. Thus, our findings reinforce the strong importance of genetics on the susceptibility to disc degeneration.

The contribution given by VDR *FokI* polymorphism for the disease may be explained by a structural modification of the VDR protein due to a change on the site starting exon 2 transcription. The F allele does not have the first ATG starting site; therefore, transcription starts at the second site, producing a shorter protein in three amino acids. Oppositely, this does not happen with the allele f; therefore, the resulting protein has a normal size. Jurutka et al.[7] showed that the smaller allele, with 424 amino acids (allele F) interacts more efficiently with the transcription factor TFIIB, leading them to the conclusion that this allele presents a higher transcriptional power, a fact that was also corroborated by Chen et al. [17] and Uiterlindem et al.[6]. The activity difference ratio for both proteins seems to be correlated to the change on its ability to bond to the active form of vitamin D for, subsequently, attaching to retinoic acid receptor (RXR) and to the vitamin-D responsive element (VDRE) and, finally, activate transcription [8]. Thus, the addition of three amino acids to vitamin-D receptor's protein is able to modify the whole vitamin-D action complex, resulting in transcriptional process failure.

In addition to vitamin-D involvement on differentiation, proliferation, maturation of cartilaginous cells and its influence on proteoglycans synthesis [5], vitamin D3 was proven to participate on cell proliferation and growth by stimulating the insulin-dependent growth factor I (IGF-I) and its receptor[18]. Admittedly, vitamin D3 activates target-genes promoter through multiple-molecules complex. It is suggested that vitamin D3 activates the IGF-I gene promoter located near to the gene for vitamin D receptor. IGF-I is expressed on intervertebral disc tissue and stimulates proteoglycans synthesis on pulpous nucleus cells [5]. Thus, changes on vitamin-D receptors expression would impair intervertebral disc's homeostasis.

Although the genetic factor has been pointed out as a major influencing factor on disc degeneration process, other factors seem to influence changes on normal metabolism of the disc [4]. From these, we could mention environmental factors, represented by physical and occupational activities, as well as lifestyles (such as diet and tobacco use), among others, although Videman e Battié[19] had concluded that the environment could explain only a small portion of this disease. Our findings seem to be consistent with this idea, once no correlation was found between patients' occupational activities and positive signs of degeneration.

Another environmental factor has been correlated to spinal conditions in many epidemiological studies [20]. Obesity is common among patients submitted to orthopaedic surgical procedures, among others, the removal of a herniated disc. However, Leboeuf[21], when conducting a review on this matter found that only 21 among 65 studies showed a positive correlation between overweight/ obesity and spinal conditions. Similarly, our findings showed no such correlation, even when the presence of allele f was compared to overweight in patients and control subjects (P=0.688).

Smoking habit is a further risk factor to be added to the list of elements suspected to have deleterious effects on intervertebral disc. Many

Personal History	Patient				Control				
	F/F		_/f		F/F		_/f		
	N	%	N	%	N	%	N	%	P*
Smoking habit	16	80.0	4	20.0	6	40.0	9	60.0	<0.001
Overweight**	1	10.0	9	90.0	18	85.7	3	14.3	0.688
Familial history	3	10.0	27	90.0	30	100.0	0	0.0	<0.001

*Test = logistic regression; ** Overweight: BMI=25 - 30kg/m².

Table 3 – Genetic polymorphisms for VDR-FokI and personal history in patients with disc degeneration and in control subjects.

VDR Genotype	Onset of Disease (age-years)					
	General	SD***	Presence of FH**	SD***	Absence of FH**	SD***
FF	38	11,9	33	11,7	41	13,3
/f	26	10,2	26	10,5	28	7,8
P*		0,013		0,025		

P*=t-test;** Familial History (FH);*** Standard Deviation (SD)

Table 4 – Correlation between age at the onset of disease and familial history in patients with disc degeneration.

Genotype	Severity*						Injury Level**			
	1		2		3		1		2	
	N	%	N	%	N	%	N	%	N	%
F/F	4	7.7	26	50.0	0	0.0	4	7.7	2	3.9
_/f	4	7.7	3	5.8	15	28.8	15	28.8	31	59.6

P*=0.0012 (Mann-Whitney's test); P**=0.175 (Fisher's test)

Table 5 – Correlation between VDR-FokI Polymorphism, severity of disc degeneration, and injury level.

investigations have documented the increasing incidence of lumbar and sciatic pain, as well as disc degeneration in chronic smokers compared to non-smokers [22]. Experiments conducted in isolated cells of the pulpous nucleus reported that cigarette components, in addition to reduce disc vascularization, promote both a decreased glycosaminoglycans production and the expression of type-II collagen gene. Other aspects associated to degeneration have been reported, such as reduced number of pulpous nucleus cells [9]. As a result of the changes on glycosaminoglycans content, the pulpous nucleus dehydrates, changing the normal disc hydrostatics and, consequently, its damping properties.

Our results showed that significantly increased frequencies of FF and reduced frequencies of -/f were preferably observed in smoker patients when compared to control subjects. In this case, it is noticed that these patients, even with the most active genotype in vitamin D capture, became more vulnerable when exposed to tobacco.

In this study, the assessment of the influence of polymorphisms in genes involved with cigarette components' metabolism - *GSTT1* and *GSTM1* – on disc degeneration has shown that 21.2% of patients and 19.3% of control subjects did not carry T1 (*GSTT1* 0/0), while for M1, nullity (*GSTM1 0/0*) among patients was 47%, and 34.1% in control subjects, with no difference between studied groups (P= 0.84 and 0.14 respectively). Nullity frequencies for genes *GSTM1* (34.1%) and *GSTT1* (19.3%) in control subjects are consistent to Rossit et al.[23].

Our results show that the allele f can be considered as a risk factor for the early onset of IDD signs. Furthermore, the smoking habit seemed to interfere on the process, regardless of the presence of a favorable genotype for genes involved in the detoxification of smoke components.

CONCLUSIONS

1. The allele f for vitamin D receptor (VDR-*Fok1*) is associated to intervertebral disc degeneration, preferably in patients with familial history of the disease; however, this does not occur with genetic polymorphisms for glutathione-S-transferase enzymes (*GSTT1* and *GSTM1*).
2. The smoking habit has shown to be a risk factor, even for patients with the FF genotype of VDR-*Fok1*, polymorphism, regardless of the genotypes for detoxification genes (*GSTM1* or *T1*), which in conjunction with individual characteristics, including overweight, familial history and severity of the disease do not show correlation. On the other hand, disease severity and its early onset are correlated to genotypes _/f.
3. The familial history for disc degeneration is reported to be a strong risk factor among patients, suggesting genetic susceptibility to the disease, with the same not occurring concerning anthropometric, environmental and occupational factors.

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