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ORIGINAL ARTICLE

Exploring the link between VDR rs2228570 and uterine leiomyoma in Iranian women

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Abstract Evidences are growing toward an inhibitory role of vitamin D3 in uterine leiomyoma pathogenesis. Uterine leiomyoma commonly affects women of reproductive age and is referred to as one of the most common indications for hysterectomy, worldwide. The effects of vitamin D are mediated through the vitamin D receptor. A single nucleotide polymorphism of the VDR gene results in longer protein with decreased activity. The present study has focused on the distribution of FokI polymorphism in Iranian patients with uterine leiomyoma. Using the PCR-RFLP method 45 cases and 53 controls were assessed. The results demonstrated a correlation between VDR TT genotype and uterine leiomyoma by Odds Ratio of 1.886 (CI, .767–4.640). The examination of heterozygous CT genotype also showed the same result by Odds Ratio of 1.875 (CI, .629–5.590). This study lends support for an increased risk of uterine leiomyoma associated with the VDR rs2228570 polymorphism. To the best of our knowledge this is the first report regarding VDR FokI polymorphism in leiomyoma patients.
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1. Introduction

Evidences are growing toward an inhibitory role of vitamin D3 (1,25(OH)2D3) in uterine leiomyoma [1,2].

Uterine leiomyoma which commonly affects women of reproductive age originates from uterine smooth muscle cells [3]. This myometrial non-cancerous tumor develops in an estrogen/progesterone dependent manner [4]. The main compliant of the patients is excessive uterine bleeding although; symptoms depend on the location of the myoma and its size [3]. It is noteworthy that leiomyoma is referred to as the most common indication for hysterectomy, worldwide [5]. Twin's and family studies revealed a genetic mechanism underlying leiomyoma [6]. It has been shown that in families with two or more leiomyoma patients, first-degree members had 2.2 times more chance to develop the disease [7].

In vitro studies revealed that vitamin D has antiestrogenic/anti progesteronic effect on fibroid cells [2]. The effect of vitamin D is mediated via the vitamin D receptor (VDR) which belongs to the nuclear receptor super family [8]. The *VDR* gene is mapped on the long arm of chromosome 12 (12q12–14) with 9 exons [9]. A single nucleotide polymorphism (SNP) of the *VDR* gene, rs2228570 (FokI), was linked to various pathological situations such as breast cancer [10]. FokI is a variable site

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located on exon 2 and presenting a C/T transition (ACG to ATG) at the translation initiation site. This transition results in three amino acids longer protein with a poor response to vitamin D [11].

Using Western blot analysis, it has been reported that myomas express low levels of VDR compared to the adjacent normal myometrium, suggesting VDR as a risk factor for myoma pathogenesis [12]. Since FokI polymorphism results in a longer protein with reduced activity; the present study has focused on the distribution of FokI polymorphism in Iranian patients suffering from uterine leiomyoma.

2. Subjects and methods

A total of 45 unrelated uterine leiomyoma patients participated in the study. They were visited by an expert gynecologist and subjected to imaging and laboratory tests. Diagnostic criteria were based on the standard international guidelines. In a case-control design 53 volunteer healthy women without a family history of myoma were considered as controls. A questionnaire was applied to interview and a written informed consent approved by the ethics committee of the Tarbiat Modares University was obtained from each participant. Work has been carried out in accordance with The Code of Ethics of the World Medical Association of Helsinki for experiments in humans.

DNA was extracted from 5 ml peripheral blood of patients and normal subjects by salting out methods and stored at −20 °C. The concentration and quality of the DNA was measured using NanoDrop® ND-1000 spectrophotometer at 260 and 280 nm. Fresh DNA working solutions (10–40 ng/μL) were prepared immediately before experiments. Specific PCR primers were designed and verified using SNPs database and BLAST website as follows:

Forward, 5′- CTGGCACTGACTCTGGCTCT
Reverse, 5′- GGGCTCACCTGAAGAAGCCT

PCR was performed by an initial denaturation step for 5 min at 94 °C then 30 amplification cycles of: denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 30 s. Final extension was allowed to proceed for 5 min at 72 °C.

FokI genotyping was conducted by restriction fragment length polymorphism (RFLP). PCR products were digested as recommended by the manufacturer's instruction (Fermentas, Cinaclon, Iran). T allele was not cleaved and presented a unique 204 bp band, while the C allele yielded 156 and 48 bp products detected by electrophoresis on 2% agarose gel.

The collected data were loaded on statistical analysis software SPSS V.16. Data were analyzed using conditional logistic regression along with 95% confidence intervals (CIs) in a 2 × 2 table. Chi-square test was selected to weigh up the null hypothesis (H0) among the groups. The *p*-values less than 0.05 were considered to be statistically significant.

3. Results

Subjects mean age was 39.07 ± 12.82 vs. controls with a mean age of 36.60 ± 13.21 years. The mean gravidity of patients and controls were 1.98 ± 1.07 and 2.03 ± 1.32, respectively. Data revealed that most of the lesions are intramural leiomyomas. In addition abnormal uterine bleeding was the most common chief complaint of the patients following low back pain.

PCR products of all samples showed a unique band with expected 204 bp on gel electrophoresis (Fig. 1).

As shown in Fig. 2, the rs2228570 SNP desired fragments were revealed by RFLP on gel.

The VDR rs2228570 SNP allele frequencies were indicated in Table 1. The minor allele (T) frequency was 0.33 and 0.43 in controls and cases, respectively. The T allele represents ATG to ACG alteration, which encodes the translation-initiation codon of VDR mRNA. As a result, protein translation initiates from upstream ATG codon. TT genotype was detected in 22% of cases vs. 17% of controls. Statistical analysis uncovers a correlation between VDR TT genotype and uterine leiomyoma by Odds Ratio of 1.886 (CI, .767–4.640). The examination of heterozygous CT genotype also demonstrated the same result by Odds Ratio of 1.875 (CI, .629–5.590). Results showed no major deviation from the expected Hardy–Weinberg equilibrium.

4. Discussion

Vitamin D levels have recently been related to the development of uterine leiomyomas, with examinations proving that lower 1,25(OH)2D3 correlates to a higher risk and larger myomas [13].

Radioimmunoassay of plasma vitamin D revealed that levels > 20 ng/ml were associated to a 32% reduced risk of myoma. The researchers also found that those who reported spending more than one hour outside (getting sun exposure) per day had a 40% decreased risk of the disease [2].

The inhibitory role of vitamin D supplementation in the treatment of myomas has also been found in animal studies. It has been reported that in rats, intravenous vitamin D3 inhibited the proliferation of fibroid cells and shrank myoma tumors [14].

Recently, Halder and colleagues discovered that uterine leiomyoma express reduced levels of VDR compared to the adjacent normal myometrium. They also suggested 1,25(OH) 2D3 as an effective, safe, nonsurgical treatment option for human uterine myomas [12].

Furthermore, in uterine leiomyoma an inverse correlation between levels of VDR and up-regulated ER-α, PR-A, and PR-B was observed suggesting that 1,25(OH)2D3 functions as an antagonist of sex steroid hormone receptors in this tissue [15].

VDR gene polymorphisms have been shown to be involved in several cancers including breast and colon. Two comprehensive case-control studies correlate rs2228570 TT genotype to a higher risk of breast cancer [10,16]. But some others have not supported this correlation [11,17].

In vitro study provided evidence that rs2228570 CC genotype enhances VDR gene expression by interaction with transcription Factor IIB [18].

Besides *VDR* gene, the polymorphisms of the genes involved in vitamin D metabolism and skin pigmentation including rs12800438 and rs6058017 were also significantly associated with leiomyoma [19].

In the present study, SNP rs2228570 was genotyped in a case/control design in Iranian leiomyoma patients. Given the fact that vitamin D functions are mediated via VDR,

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