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Brief communication

Case-control study of vitamin D receptor gene polymorphism in Pakistani rheumatoid arthritis patients



Estudo caso-controle do polimorfismo do gene receptor da vitamina D em pacientes paquistaneses com artrite reumatoide

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Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory disease, characterized by autoantibody formation and progressive joint destruction. In RA, synovial membrane primarily gets affected but it can affect other organs as well.¹ RA is a devastating and common disease having prevalence of approximately 1% in world population, and 0.14–0.3% Pakistani population, with women affected three times more often than men.² The exact etiology of RA is yet unknown but it depends on the interaction of number of environmental and genetic factors.³ In recent years starring role of vitamin D, a secosteroid hormone carries out the activities through VDR

and VDR itself has been found in immunomodulation, thus we selected the gene VDR to investigate its impact in RA susceptibility. The VDR protein is translated from VDR gene which is extremely polymorphic. The four important VDR gene variants, rs2228570 in exon 2, rs1544410, rs7975232 in intron 8 and rs731236 in exon 9 have been widely studied and are also known to be associated with autoimmune diseases including systemic lupus erythematosus, Addison's disease, type I diabetes and RA.⁴

Study of differences in genotype distribution would be helpful to identify the consequences of ethnic allele variation because of distinct genetic backgrounds, additionally have unlike environmental elements. It also helps in interpreting and detecting phenotypic variability in terms of disease

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Table 1 – General characteristics of population studied.

Characteristics	Cases (n = 100)	Control (n = 100)
Male	37	31
Female	63	69
Age (mean)	44.2	43

severity. Based on genotype distinctions, biological interpretation could be claimed by disease association studies. Polymorphism rs1544410 located in intron 8 near 3' UTR region, affects the VDR gene transcription level, transcript stability and post transcriptional modifications.⁵ Some previously reported data showed functional significance in the distribution of rs1544410 genotype, our study was accompanied to evaluate the association of VDR polymorphism with RA in our group of study. There is a great need to discover the potential genetic risk markers of RA susceptibility, in our Pakistani population, but no such work have previously been done for Pakistani RA patients.

Materials and methods

A total of 100 patients and 100 healthy controls (non-symptomatic individuals) matched for age and sex (Table 1) were recruited for this case-control study. Patients fulfilling the 2011 classification criteria of the American College of Rheumatology (ACR-2011) were diagnosed by rheumatologists from Rehmat Noor Clinic, Rawalpindi, working in collaboration with our research group. The study was approved by Institutional Review Board (IRB) of ASAB-NUST, following the rules of the Declaration of Helsinki and the informed consent was taken. Blood samples of all the included RA patients as well as from the healthy controls were collected in 0.5 M EDTA (ethylenediaminetetraacetic acid) tubes. SNP that causes single nucleotide change from major allele frequency guanine (G) to minor allele frequency adenine (A) was investigated to check the presence of polymorphism in our study group.

Genomic DNA was extracted from collected blood samples using phenol-chloroform method, quantified by nanodrop (eppendorf Biophotometer Plus). Manually designed two forward and a common reverse primer (VDR-F1-5'GCCACAGACAGGCCTGCG3', VDR-F2-5'GCCACAGACAGGCC-TGCA3', VDR-R-5'GTCACTGCACATTGCCTCAA3') were used for VDR gene non-coding variant (rs1544410) genotyping through Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). PCR products were

resolved on 2% agarose gel after amplification in 96 wells thermocycler 2720 (Applied Biosystems). Statistical analysis was performed using Graphpad Prism 6V software, Chi square (X^2) and two-tailed Fisher's exact tests were applied for association analysis of rs1544410 polymorphism in our study population. Odds ratio (OR) with 95% confidence interval was calculated and p value <0.05 was considered statistically significant.

Results

Hardy-Weinberg equilibrium (HWE) calculations made for control group shows no significant difference between observed values and the expected values, with the p -value of 0.0034 and showed to be in HWE. This indicates that there was no significant drift in observed allele frequencies of patients, when compared with controls, thus both the groups were suitable for further analysis and association studies. The frequency of all possible genotypes did not also show any significant difference in patients and healthy individuals (Table 2 and Fig. 1A). The value of X^2 was found to be 5.662 with a probability of error (p -value) of 0.0590. Allele frequencies for RA patients were also not significantly different from controls (Table 2 and Fig. 1B). The observed results indicated no significant association between the SNP and RA. Thus, the data nullified any significant association of VDR gene polymorphism with RA in our group of study. Sex specific association with rs1544410 was also carried out but no significant association has been shown between RA affected females and males (data not shown).

Discussion

In the recent years gene polymorphism has been one of the utmost discussed topic of genomic variations. RA is a systemic inflammatory disease that affects the bone and cartilage of the patient. Vitamin D exerts several immunomodulatory effects and thus may play a role in the course of autoimmune diseases.⁶ A considerable association between VD insufficiency and an increased occurrence of autoimmune disorders has been determined.⁷ It has been observed that in RA patients lymphocytes express VDR.⁸ In cytoplasm hydroxylated form of vitamin D 1,25[OH₂]D binds to VDR which then move toward nucleus, where it increases the VD dependent genes transcription essential in calcium and bone metabolism,⁹ also inhibiting T-cell proliferation and the release of Th1 cytokine

Table 2 – Genotype and allele frequencies distribution in cases and controls.

Genotype	Genotype frequencies		X^2 statistics			Allele	Allele frequencies		Statistics	
	Cases (n = 100)	Control (n = 100)	X^2 value	df	p-value (alpha <0.05)		Case (n = 100) (%)	Control (n = 70) (%)	Odds ratio (OR) (95% CI)	p-value
GG	31.0	40.0	5.662	2	0.0590	G	56.0	66.0	0.6556 (0.4375–0.9825)	0.0513
GA	50.0	52.0								
AA	19.0	8.0								

df, degree of freedom.

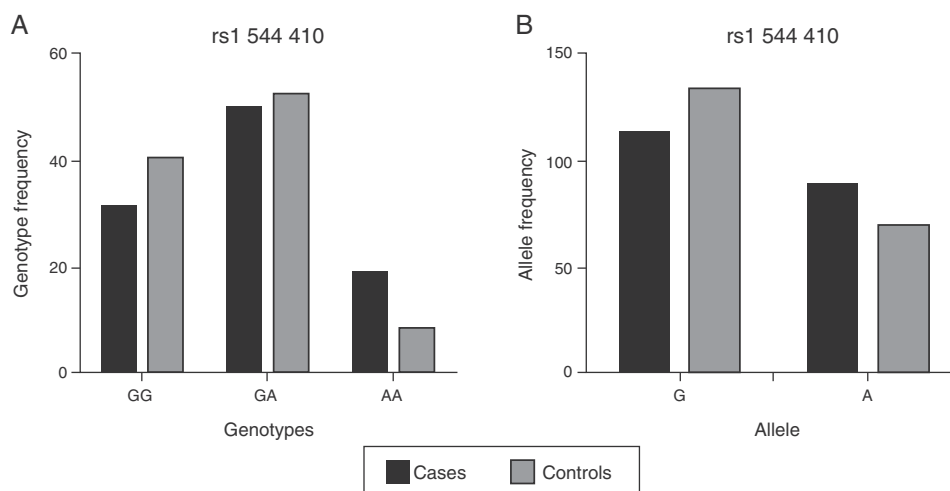


Fig. 1 – A, Graph showing genotype distribution of VDR polymorphism (rs1544410) in RA cases and healthy controls. B, Graph showing allelic distribution of VDR polymorphism (rs1544410) in RA cases and healthy controls.

such as IL-2, IFN- γ and TNF- α .¹⁰ Various studies have shown that the risk of autoimmune diseases have been increased due to vitamin D deficiency, additionally a considerable clinical improvement was observed in the VD-treated RA patients.¹¹ In our study VDR gene rs1544410 polymorphism investigation shows no statistically significant differences in genotype and allele frequencies of RA patients and healthy controls. Our observations are in consistence with the findings of Maalej et al.¹ who demonstrated no association of the rs1544410 polymorphism with the development of RA in French and Tunisian populations. However, this polymorphism was associated with other autoimmune diseases like type 1 diabetes (T1D) in many countries, including different populations like Hungary, Japan, Greece, Bangladesh, Taiwan and Chile.¹² Significant association was also found between rs1544410 polymorphism and osteoporosis.¹³ Furthermore, the studies have been conducted on other various autoimmune diseases including lupus, cirrhosis, hepatitis, Crohn's, Graves disease and multiple sclerosis in relationship with rs1544410 polymorphism and the incidence of systemic lupus erythematosus in Japanese and Chinese was found. So the polymorphism can be investigated in association with other immune diseases.¹⁴ Polymorphisms in VDR gene have been observed to be associated in a sex dependent manner, i.e. more prevalent in females affected with RA.¹⁵ Sex specific association with rs1544410 in our group has indicated that polymorphism does not effect in sex dependent manner in case of RA and there is no link of this polymorphism to the increased incidence of RA among females.

Although finding of this study shows no evidence of rs1544410 association with RA in our group of study, but there might be other polymorphisms found that would have been recognized as risk factors for RA. There is a need to investigate other molecules implicated in the inflammatory pathway of RA for genetic association with RA pathogenesis. Other polymorphisms in the VDR gene may have significant association toward RA, so larger population study is required to delineate the association.

Conflicts of interest

The authors declare no conflicts of interest.

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