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GENETICS

Association between a PD-1 gene polymorphism and antisperm antibody-related infertility in Iranian men

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Abstract
Objective Programmed cell death-1 (PD-1, Pdcd1), an immunoreceptor belonging to the CD28/CTLA-4 family negatively regulates antigen receptor signalling by recruiting protein tyrosine phosphatase, SHP-2 upon interacting with either of two ligands, PD-L1 or PD-L2. This study investigates PD-1 gene polymorphism in patients with antisperm antibody-related infertility
Methods Genotyping was performed by polymerase chain reaction and restriction enzyme digestion (PCR-RFLP), this polymorphism was genotyped in 145 Iranian subjects (61 patients with antisperm antibody-related infertility and 84 healthy controls).
Results Patients frequencies of the G/A genotype in comparison with healthy controls (38.2 % vs. 32.7 %, OR =1.21, P=0.35) were not significantly different. However, G/G and A/A genotype frequencies between patients and healthy controls were significantly different (P=0.042, P=0.00001, respectively). Also, allele frequencies of this polymorphism were significantly different (P=0.0012) in patients compared to healthy controls.
Conclusion According to these results, there is a correlation between PD-1 gene polymorphism and susceptibility to antisperm antibody-related infertility in our study group.

Keywords PD-1 · Polymorphism · Antisperm antibodies · Infertility

Capsule Anti-sperm antibodies (ASA) production is a result of an abnormal immune condition and can cause infertility in men. We found a significant correlation between PD-1.3 genotypes and risk of ASA-related infertility. These findings raises interests for further investigation to the mechanism of PD-1/PD-1 ligand interaction in the development of ASA.

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Introduction

Infertility remains a threat to successful reproduction by couples desirous of pregnancy and is estimated to affect one out of five couples worldwide [1]. In about 10–20 % of the cases, no definitive cause has been identified. 9–36 % of the cases have been attributed to the production of anti-sperm antibodies (ASAs) in men. The presence of these antibodies was first reported by Samel R. Meaker (1922) [2].

ASAs are detected in blood, semen and follicular fluid as well as cervicovaginal secretions and appear to hinder sperm movement, capacitation and fertilization that ultimately inhibit embryo implantation [3]. ASAs develop against some sperm antigens such as nuclear auto-antigenic sperm protein (NASP) (a histone-binding protein) and affect fertility rate [4].

T lymphocytes with the αβ- and γδ-antigen receptors are present in ASA bearing semen. Moreover, the presence of ASAs was associated with increased numbers of both γδ and cytotoxic T cells in seminal fluid of male with unexplained

infertility [5]. Traces of αβ- and γδ T cell subsets and antisperm antibodies might be detectible in semen of fertile men in some cases [6, 7]

The presence of antigen specific T cells in semen fluid suggest their implication in the development of local inflammation and development of auto-antibodies. As normally that the peripheral tolerance pathways locally implicate in protection against auto-reactive responses. Programmed death 1 (PD-1), a CD28 family member and inhibitor of cellular activation of T and B cells long known to actively participate in maintaining peripheral tolerance via activation of immunoreceptor tyrosine-related inhibitory motif (ITIM) pathway [8].

The immunoinhibitory function of PD-1 was supported by the observation that mice deficient in PD-1 expression developed autoimmune diseases, despite having distinct phenotypes on different genetic backgrounds [9]. To date, more than 30 SNPs have been identified in human PD-1 gene [10, 11]. Prokunina et al. reported that the allele A of a SNP named PD1.3 (PD1.3A) in intron 4 is associated with the development of SLE in Europeans (relative risk=2.6) and Mexicans (relative risk=3.5) [12]

To date, Single Nucleotide Polymorphism association with PD 1.3 has been reported in several auto-antibody associated autoimmune diseases; including psoriasis [13], rheumatoid arthritis [14], Type I diabetes [11], Multiple sclerosis [15], Ankylosing spondylitis [16] and Allergy [17].

Materials and methods

Subjects

A total of 61males with primary ASA-related infertility attending Avicenna Centre of Infertility and in vitro Fertilization (Tehran) were enrolled in this case–control study. They were clinically examined and their ASA-related infertility was evaluated by the consultant medical staff of the mentioned centre. All The patients were Iranian and aged 27.3±0.9 years (mean S.E.). In addition, 84 fertile ethnicity matched males, aged (28.2±1.2 years) and without a history of personal or familial infertility or any other autoimmune disorders were included in the study, as control.

Blood samples were collected from subjects between 2013 and 2014. Each subject provided written informed consent to participate in the study. The study was approved by the Ethics Committee of Avicenna Centre of Infertility and in vitro Fertilization

DNA extraction

Peripheral venous blood samples were collected from the subjects in EDTA coated tubes. The genomic DNA was extracted by salting out method, using Relax Gene Blood DNA System (TIANGENBIOTECH, Beijing, China), according to the manufactures' protocol. DNA purity and concentrations were confirmed by spectrophotometric measurement of absorbance at 260/280 nm.

Determination of PD-1 genotype

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was utilized to determine the PD-1 polymorphism. The PCR primers were designed using Primer Premier 5.0 software. For PD-1.3, the primer sequences were 5 -CCCCAGGCAGCAACCTCAAT-3 (forward) and 5 GACCGCAGGCAGGCACATAT-3 (reverse). Amplification was performed under the following conditions: An initial denaturation for 5minat 95°C, followed by denaturation at 95°C, annealing at 56°C, and extension at 72°C for30 s, followed by a final extension at 72°C for 7 min. A final volume of 25 μL of extract was used to detect the PD-1.3 polymorphism. Subsequently the amplified PCR products were digested using restriction enzyme PST I, subjected to gel electrophoresis and monitored by ethidium bromide.

Statistical analysis

Cases and controls were tested for Hardy-Weinberg equilibrium (HWE) separately. Allele and genotype frequencies in patient and control subject were calculated by direct gene counting. Statistical evaluation was carried out using the Statistical Package for the Social Sciences (SPSS) version 18. Chi square test and Fisher's exact test were used to compare frequencies of alleles/genotypes in cases and controls. Odds ratios and 95 % confidence intervals (CIs) for relative risks were calculated. A probability value of $P<0.05$ was considered as statistically significant and all reported P values were two-tailed.

Results

In our study the distribution of genotype frequencies in control groups was consistent with HWE. AA and GG genotype frequencies between the patients and healthy controls are significantly different ($P=0.00001$, OR=0.62, 95 % CI=0.22-078, $P=0.042$, OR=1.74, 95 % CI=0.1-3.32, respectively).

Moreover, the allele frequencies of the studied polymorphism were significantly different in patients vs. healthy control group ($P=0.0012$, OR=0.74, 95 % CI=0.32-0.71). A lower frequency of GG and GA and higher frequency of AA genotypes in the patients vs. controls were observed (29.6 %, 32.7 %, and 37.7 in patients vs. 41.6 %, 38.2 %, and 20.2 %in controls, $P=0.042$, $P=0.35$, and $P=0.00001$, respectively). A significantly higher frequency of A allele in patients vs. control group has been observed as well (55.8 in patients vs. 39.1 % in controls, $P=0.0001$) (Table 1).

Table 1 Genotype and Allele Frequencies of PD.1.3A/G single nucleotide polymorphism (SNP)in Patients with ASA-related infertility and Healthy Controls

Gene	Genotype	Normal %(No)	Infertile % (No)	P value	OR	95% CI
PD.1.3A/G	AA	20.2 (17)	37.7 (23)	0.00001	0.62	0.22-078
	GA	38.2 (32)	32.7 (20)	0.35	1.21	0.61-2.11
	GG	41.6 (35)	29.6 (18)	0.042	1.74	0.1-3.32
	A allele	39.1 (66)	55.8 (86)	0.0012	0.74	0.32-0.71
	G allele	61.9 (102)	44.2(68)			

Discussion

We studied the correlation between PD-1.3 genotypes and risk of ASA-related infertility in an Iranian group of infertile patients, at the level of single nucleotide polymorphisms (SNP) of the genome and consequently assessed their association with prognostic factors. A meaningful association between male ASA-related infertility and PD-1 gene polymorphism has been observed in this study, suggesting a correlation between impaired PD-1-associated immunomodulation to the development of ASAs. To our knowledge this is a novel study that signifies the role of PD-1 in the maintenance of immune-tolerance in this setting.

Previous studies have shown that PD-1 gene polymorphisms are correlated with autoimmune diseases such as ankylosing spondylitis [17], rheumatoid arthritis [10], and systemic lupus erythematous [20]. Moreover PD 1.3 SNP has been reported in different autoimmune settings, including rheumatoid arthritis [14], Type I diabetes [11], Multiple sclerosis [15], Ankylosing spondylitis [16] and Allergy [17].

The cross-reactivity of ASAs with other auto-antibodies, including antithymocyte antibody, antinuclear antibody, antiphospholipid antibody, demonstrated in other studies [19], suggesting a polyclonal B-cell activation, similar to that seen in other autoimmune diseases (e.g. lupus erythematous) might implicate in the development of ASA-related infertility.

The mechanism behind the polyclonal B and T cell overactivation in autoimmune conditions is an ongoing challenge that still remained elusive. PD-1 is an inducible receptor expressed on different immune cell types, including CD4+ T cells, CD8+ T cells, NKT cells, B cells, and monocytes [20]. PD-1: PD-1 ligand interactions inhibit positive selection during the double negative to $CD4^+$ $CD8^+$ (DP) maturational stage [21]. PD-1 also implicates in negative selection [22], and is crucial for regulation of self-reactive T cells that escape negative selection in the periphery [22]. Studies in mouse models of autoimmune disease have revealed that PD-1: PD-1 ligand interaction not only is important in the inhibition of the primary phase of T cell activation and expansion, but also modulates T cell effector functions upon antigen reencounter [25]. Together, these are indicating the key role of PD-1/PD-1ligand ligand interaction in the maintenance of central and peripheral tolerance.

An increase in the number of αβ and γδ T cell lineage in semen of infertile men with antisperm antibodies [24] suggests that a local autoimmune T cell-mediated immune reaction toward spermatozoids might be responsible for the elimination of these cells in reproductive tract [6], and the up regulation of PD-1 upon activation of T cells could potentially reverse this effect. Recent study by Valentina Dal Secco et al. demonstrated that mouse testis sertoli cells express elevated level of PD-1 ligand in vitro, by IFN γ treatment [25]

To our knowledge, our study is the first investigation of the contribution of PD-1 in male ASA-related infertility in human subjects. This study raises more interest for further investigation to the mechanism of PD-1/PD-1 ligand interaction in the development of ASA-related infertility.

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