کارگاه های آموزشی مرکز اطلاعات علمی چهاد دانشگاهی

مباحث پیشرفته یادگیری عمیق؛ شبکه های نویه گرافی
(Graph Attention Networks)

کارگاه آنلاین آموزش استفاده از وب آو ساینس

کارگاه آنلاین مقاله روزمره انگلیسی
Original Article

Tumor Necrosis Factor Alpha -308 G/A Single Nucleotide Polymorphism and Risk of Sperm Abnormalities in Iranian Males

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Abstract

**Background:** Signaling molecules such as cytokines regulate spermatogenesis during the maturation of germ cells and sperm apoptosis. Tumor necrosis factor alpha (TNFα) is one of the most-documented cytokines that is involved in spermatogenesis. We investigated the association of the TNFα -308 G/A single nucleotide polymorphism with sperm abnormalities in Iranian males.

**Materials and Methods:** This case-control study included 180 infertile men who referred to Yazd Research and Clinical Center for Infertility and 100 healthy normospermic controls. Infertile men were classified into four groups ofazoospermia (n=91), oligospermia (n=26), teratospermia (n=30) and asthenoteratospermia (n=33). After sperm analysis, DNA was extracted from blood and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was carried out for the genotyping of TNFα-308 G/A.

**Results:** The A allele was significantly associated with sperm abnormality in our population [(P<0.001, odds ratios (OR) 95% confidence interval (CI)=2.31). In addition, the A allele was also associated with azoospermia (P<0.001, OR (95% CI)=2.484), oligospermia (P=0.005, OR (95% CI)=2.51) and teratospermia (P<0.001, OR (95% CI)=3.385) but not with asthenoteratospermia (P=0.623).

**Conclusion:** Our data suggest that this single nucleotide polymorphism (SNP) maybe associated with the risk of sperm abnormality in infertile men of Iranian origin.

**Keywords:** Infertility, Cytokines, Tumor Necrosis Factor Alpha, Polymorphism, Restriction Fragment Length Polymorphism

Introduction

It is estimated that about 15% of couples globally suffer from infertility. Male infertility constitutes 50% of causes among which genetic factors are mainly responsible (1, 2). Other causes of male infertility maybe related to post-testicular obstruction, endocrine dysfunction and vascular abnormalities (3). During the last decade, it has become clear that some signaling molecules that mediate the intercellular communication and integration have an important role in the hormonal regulation of germ cell maturation in testis. Cytokines are mediator molecules that are involved in this regulation and as a result have an important impact on spermatogenesis (4, 5). Among cytokines, tumor necrosis factor alpha (TNFα) is not only the
most studied molecule but also the most potent in
germin cell apoptosis, peritubular cell secretion and
regulation of spermatogenesis (6). Its receptors
are present in Sertoli and Leydig cells, allowing
TNFα to regulate secretion from these cells (7).
Some studies have shown a negative association
of TNFα plasma levels with sperm motility and
morphology (8, 9). The effect of TNFα on testos-
erone production, which has a direct impact on
male infertility, has also been reported in some ex-
perimal models (10, 11).

The TNFα gene as a single copy gene is located
on chromosome 6p21.3 within the major histocom-
patibility complex (MHC) gene cluster (12). Gene
variation such as single nucleotide polymorphisms
(SNPs) in TNFα gene can alter TNFα production.
Several SNPs including -308 G/A, -1031 T/C, -863
C/A, -857 C/T, -575 G/A, -244 G/A and
-238 G/A in the promoter region of the gene have
been investigated (13). The -308 G/A SNP in the
promoter region of TNFα has been implicated to
increase promoter activity, leading to an increased
production of TNFα in blood (14, 15). Some studies
have reported a negative association of TNFα with sperm motility and morphology (9, 16, 17).
Zalata et al. (18) showed the association of the
-308 G/A SNP with increased seminal caspase-9
and decreased sperm motility, count, morphology,
acrosin activity and seminal α-glucosidase. Shukla
et al. (19) showed that there is a strong association
between this SNP and male infertility in the Indian
populations of Uttar Pradesh.

In this study, given that TNFα is an important
regulator of steroidogenesis and may affect sper-
matogenesis, we investigated the association of the
TNFα -308 G/A SNP with different kinds of sperm
abnormality in infertile males of Iranian origin.

Materials and Methods

This case-control study included 180 infertile
males as the case group and 100 healthy nor-
mospermic individuals as the control group. The
case individuals were recruited from Yazd Res-
search and Clinical Center for Infertility from Sep-
tember 2012 until August 2013. They were divid-
ed based on sperm abnormality into azoospermia
(n=91, AZ group), oligospermia (n=26, OL group),
teratospermia (n=30, T group) and asthenoterato-
spermia (n=33, AT group) groups. This study was
approved by the Ethics Committee of Shahid Sad-
oughi University of Medical Sciences, Yazd, Iran.
Written informed consent was obtained from each
individual. All semen analysis and clinical exami-
nations were done according to the World Health
Organization guidelines (20).

Tumor necrosis factor alpha -308 polymor-
phism genotyping

Genomic DNA was extracted from whole
blood samples using the salting out method. We
used the polymerase chain reaction-restriction
fragment length polymorphism (PCR-RFLP)
method for genotyping of TNFα -308G/A. The
F: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and
R: 5′-TCCTCCTCTGCTCCGATTCCG-3′ prim-
ers were used to amplify a107 bp fragment of the
TNFα promoter that included this SNP. PCR was
carried out in a total volume of 25 μl containing
3-5 μl genomic DNA, 1 μl of each primer (10 μM)
and 12.5 μl of PCR Master Mix (Cinnagen, Iran)
and dH2O. The condition of DNA amplification
was an initial denaturation step at 94°C for 5 min-
utes, followed by 35 cycles of 94°C for 40 sec-
onds, 60°C for 1 minute and 72°C for 40 seconds,
and a final extension step at 72°C for 5 minutes
and hold at 4°C. Subsequently, the PCR products
were digested with NcoI restriction enzyme (14
hours at 37°C) and the specific bands were identi-
ified using 2% agarose gel electrophoresis in 1X
Tris/Borate/EDTA (TBE) buffer and visualized
under the ultraviolet (UV) light. When digested,
the PCR fragment was cleaved into two fragments
with sizes 87 bp and 20 bp.

Statistical analysis

The frequency of alleles and genotypes were com-
pared with a 2×2 contingency table using Chi-
squared and Fisher’s exact test. Fisher’s exact test
was used when sample sizes were small in each
category. We considered P<0.05 as a statistically
significant and 95% confidence interval (CI) for
calculating odds ratios (OR). We used the SPSS
statistical software (version 20, SPSS Inc., Chi-
icago, IL, USA) for all statistical analyses.

Results

In this study, PCR-RFLP was able to identify both
alleles efficiently at position -308 in the promoter
region of TNFα gene (Fig.1).
Table 1 shows the related parameters of each group. The frequencies of alleles and genotypes and their association with the studied group are listed in Table 2. Ancestral genotype GG and allele G were taken as reference. Statistical analysis showed that there is a significant association between this SNP and the AZ, O and T patient groups but not with the AT group. The frequency of the AA genotype was 13% in the healthy normospermic (N) group, 27.4% in the AZ group [OR (95% CI) = 2.535], 26.9% in the O group [OR (95% CI) = 2.97], 30% in the T group [OR (95% CI) = 2.86] and 15.2% in the AT group. The AG genotype was identified in 22% of the N group, 32.9% of the AZ group [OR (95% CI) = 1.74], 34.6% of the O group [OR (95% CI) = 1.87], 43.3% of the T group [OR (95% CI) = 2.71] and 24.2% of the AT group.

Table 2: The frequencies of alleles and genotypes of the -308 G/A SNP in the TNFα promoter in the azoospermic, teratospermic, Asthenoteratospermic and Oligospermic groups

| Genotype-allele/ group | Normospermic (%) n=100 | Azoospermic (%) n=91 | P; OR (95% CI) | Oligospermic (%) n=26 | P; OR (95% CI) |
|------------------------|------------------------|----------------------|-----------------|------------------------|----------------|
| **AA**                 | 13 (13%)               | 25 (27.4%)           | 0.018; 2.535 (1.2-5.3) | 7 (26.9%)               | 0.040; 2.97 (1.076-8.22) |
| **AG**                 | 22 (22%)               | 30 (32.9%)           | 0.010; 1.74 (0.916-3.20) | 9 (34.6%)               | 0.206; 1.87 (0.736-4.787) |
| **GG**                 | 65 (65%)               | 36 (39.5%)           | Ref.             | 10 (38.5%)              | Ref.             |
| **AA+AG**              | 35 (35%)               | 55 (60%)             | 0.001; 2.837 (1.57-5.107) | 16 (61%)               | 0.024; 2.971 (1.22-7.240) |
| **A**                  | 48 (24%)               | 80 (43.9%)           | <0.001; 2.484 (1.604-3.845) | 23 (44.2%)             | 0.005; 2.51 (1.32-4.7) |
| **G**                  | 152 (76%)              | 102 (56%)            | Ref.             | 29 (55.8%)              | Ref.             |

Table 1: The sperm parameters in the case group

| Group              | Sperm parameter | Mean ± SD |
|--------------------|-----------------|-----------|
| Azoospermia        | Sperm count (10⁶/mL) | 0         |
| Oligospermia       | Sperm count (10⁶/mL) | 6.6 ± 2.3 |
| Asthenoteratospermia | Motility (grades a+b)%/Morphology (% normal forms) | 10.7 ± 5.8/4.7 ± 2.8 |
| Teratospermia      | Morphology (% normal forms) | 6.9 ± 3.4 |

TNFα: Tumor necrosis factor alpha, SNP: Single nucleotide polymorphism, OR: Odds ratios, CI: Confidence interval, ’: Significant P<0.05. Ancestral genotypes GG* and alleles G* were taken as reference.
Discussion

Genetic variation such as SNPs in TNFα promoter region may affect its expression. There are several studies that have investigated the association of TNFα SNPs with different diseases such as colorectal cancer, pre-eclampsia, prostate cancer and Crohn’s disease (21, 22). The basic knowledge about the crucial role of TNFα in spermatogenesis is based on the study by Suh et al. (23) in which male TNFα knockout mice showed delayed spermatogenesis, reduced testis weight and sperm count in comparison with wild-type mice.

In this study, we analyzed the TNFα -308 G/A SNP to identify its possible association with sperm abnormality in Iranian males. To the best of our knowledge, this study has not been undertaken in Iranian males. Our findings indicate that this SNP is significantly associated with azoospermia, oligospermia and teratospermia. In other words, this SNP is among many genetic factors that may lead to a decreased count of sperm and abnormal morphology in our cases.

Similar to our study, Tronchon et al. (13) also found a positive association of the TNFα -308 A allele with oligospermia and teratospermia. Zalata et al. (18) also observed an increased frequency of TNFα -308 GG genotypes in fertile males compared with the infertile group in the Egyptian population. In the Indian population, consistently, the frequency of the AA genotype was higher in infertile individuals rather than fertile subjects, and higher level of apoptosis and necrosis levels were observed in infertile males, likely due to increased levels of reactive oxygen species (19). In contrast, Kurz et al. (24) found no association of TNFα -308 C>T and -863 C>A SNPs with sperm abnormalities (asthenozoospermia and oligo-asthenoteratozoospermia) in the Australian population. In the Greek population, Lazaros et al. (25) also found no association between -863 C>A and semen quality. The differences between the results of studies can be related to different number of studied individuals and different studied population with subgroups and ethnicities.

Based on the results of previous studies, TNFα is known to affect spermatogenesis by changing the structure of the blood-testis barrier and apical ectoplasmic specialization of Sertoli cells, which may lead to abnormal spermatogenesis (26). Moreover, it affects the Fas ligand system and germ cell apoptosis which have important roles in the germ cell maturation and normal spermatogenesis (27, 28). Binding of the TNFα molecule with its type 1 receptor activates signaling molecules in the transduction pathway, in which adaptor proteins interact with conserved death domains. The adaptor proteins increase activation of caspase-8 causing the release of cytochrome c from mitochondria. This is followed by the configuration of a high molecular weight complex (apoptotic protease activating factor-1, cytochrome C, and caspase-9) that activates caspase-3 and causes cell death (29, 30). Our study certainly has its own limitations and further association studies with more polymorphisms and individuals may provide more representative data on the association of TNFα variation with different sperm abnormalities in the Iranian population.

Conclusion

Our study shows that there is a positive association between TNFα -308 G/A SNP and different sperm abnormalities in the Iranian population. Given that the A allele leads to increased expression of TNFα, anti-TNFα agents could be a useful treatment for male infertility.

Acknowledgements

We thank all the individuals who participated in this study. There is no financial support and conflict of interest in this study.

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