

Genetic polymorphisms of *hspa1b* and *hspa1l* in infertile men

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Abstract

Objective: To investigate the relationship between the HSP 70 gene polymorphism and primary infertility in males with normal sperm-parameters.

Methods: The case-control study was conducted in Sanliurfa, Turkey, from September 2010 to August 2011 and comprised infertile males as cases and healthy fertile controls. Deoxyribonucleic acid was isolated from the blood of both groups, and polymorphisms of the HSPA1B gene (NM_005346.4, GI: 3304):c.1059G>A, (PstI G>A; dbSNP: rs1061581G>A) and HSPA1L gene (NM_005527.3, GI: 3305) c.1478C>T (NcoI C>T, dbSNP: rs2227956) were analysed with polymerase chain reaction-restriction fragment length polymorphism technique. SPSS version 11.5 was used for statistical analysis.

Results: Of the 140 males in the study, 68 (28.5%) were infertile cases and 72 (51.4%) fertile controls. There was no statistically significant difference between GA (heterozygous) and AA (homozygous, polymorphic) genotypes of the c.1059G>A polymorphic point of the HSPA1B gene or between the A allele of the cases and controls ($p > 0.05$). There was no statistically significant difference between the CT (heterozygote) and TT (homozygous, polymorphic) genotypes of the c.1478C>T polymorphic area of the HSPA1L gene or between the T alleles of the cases and the controls ($p > 0.05$).

Conclusions: Infertility in males with normal sperm parameters was not significantly associated with HSPA1B:c.1059G>A and HSPA1L:c.1478C>T gene polymorphisms. Further prospective studies with larger sample sizes and different gene groups are required to clarify the issue.

Keywords: Infertility, HSP70, Gene, Polymorphism, PCR, RFLP. (JPMA 65: 701; 2015)

Introduction

Despite regular sexual activity, 15% of couples do not have children within the first year.¹ Male factor infertility is involved in half of those cases.¹ Approximately, in the 40-70% male infertility, even the underlying cause is known, in about 30% of these couples, no aetiology can be found, and it is considered idiopathic infertility.^{2,3} The regulation of the spermatogenesis process through gene expression is very important because deoxyribonucleic acid (DNA) damage, disruption of the cell cycle and apoptosis may result in abnormal spermatogenesis.⁴ In humans, the chromatin of the mature sperm nucleus can be abnormally packaged due to external stresses, such as oxidation or temperature elevation.⁵ There are many processes involved in protecting the cell against the harmful effects of cellular stress. One of these is the synthesis of a protein family called heat shock protein (HSP).⁶ The induction of HSPs is a critical and highly conserved cellular response, which protects cells from a range of stresses, including damage caused by normal physiological processes, extreme environmental stress or disease. They are very important in all stages of cell metabolism, including growth, differentiation, division and even cell death. The

HSP70 family is the most highly conserved of the many HSP families across a wide range of species from bacteria to plants and animals.⁷ Previous studies showed that HSPs are effective during spermatogenesis and that degradation of the HSP70-2 gene results in failed meiosis and male infertility.^{8,9} The current study was planned to investigate the relationship between the HSP70 gene polymorphism and infertility in infertile males with normal sperm parameters

Subjects and Methods

The case-control study was conducted in Sanliurfa in southeastern Anatolia region of Turkey from September 2010 to August 2011 and comprised infertile males as cases and healthy fertile controls. All the subjects were from Sanliurfa province, and attended a male infertility clinic. The study was approved by the Harran University Institutional Review Board, and informed consent was obtained from each subject. A detailed medical history was obtained from all the subjects. All the couples presenting for infertility evaluation had a minimum of one year of unprotected intercourse. The idiopathic subfertility group had normal standard semen parameters on repeated analyses and a normal genital exam. Infertile patients with well-known pathological features, such as varicocele, leukospermia, hormonal abnormalities and/or obstruction were excluded. Also excluded were those with cryptorchidism, vasectomy, abnormal liver function, cigarette smoking and alcohol consumption. Only patients with

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primary infertility with normal sperm parameters who had a fertile female partner were included. Ethylenediaminetetraacetic acid (EDTA) blood was taken from each individual, and genomic DNA was extracted from whole blood leukocytes using a standard salting out procedure.¹⁰ HSPA1 B and HSPA1 L gene polymorphisms were analysed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. In addition, information on the body mass index (BMI, kg/m²), semen volume (ml), total sperm number (million), motility rate and sperm morphology (from the medical records of the patients) were obtained for all the cases and the controls.

The polymorphic sites of HSPA1B (NM_005346.4; GI: 3304):c.1059G>A (rs1061581), and HSPA1L (NM_005527.3, GI: 3305): c.1478C>T (rs2227956) were investigated with the PCR-RFLP technique. The primer sequences¹¹ used were as follows: 5'-CATCGACTTCTACACGTCCA-3' and 5'-CAAAGTCCTTGAGTCCCAAC-3' for HSPA1B: c.1059G>A and 5'-GGACAAGTCTGAGAAGGTACAG-3' and 5'-GTAAGTCTGAGTCTGG-3' for HSPA1L:c.1478C>T. The PCR reactions were performed in a 10 l of reaction volume, including 1xPCR buffer, 2mM magnesium chloride (MgCl₂), 0.2 mM of deoxynucleotide triphosphate (dNTP, Fermentas, St. Leon-Rot, Germany), 0.2 μM of each primer (BioBasic Inc., Ontario, Canada), 30ng of DNA and 0.5 unit of Taq DNA polymerase (Fermentas). The PCR programme for the two polymorphic sites was performed as follows: 94°C for 3min (initial denaturation), 30 cycles, with 94°C for 30s, 60°C for HSPA1B (for HSPA1L, 55°C) for 30s, 72°C for 30s and 72°C for 5 min (final extension).

Ten microlitres of PCR product in a 30μl volume for HSPA1B:c.1059G>A and HSPA1L:c.1478C>T were separately digested with 1.5 units of PstI and NcoI (Fermentas) at 37°C for 2h. The digested PCR products were separated on 1.5% agarose gel and analysed using the Alpha Imager System (Alpha Innotech, San Leandro, CA, USA). The digested HSPA1B:c.1059 G allele yielded two fragments of 934 and 183bp, and the A allele yielded a 1117bp fragment (Figure-1). The HSPA1L:c.1478 C allele yielded a fragment of 878bp, and the T allele yielded two fragments of 554 and 324bp (Figure-2).

SPSS version 11.5 was used for statistical analysis. Student's t-test and chi-square tests were used to determine differences in the means of demographic and clinical profiles. Genotype and allele frequencies of HSPA1B:c.1059G>A and HSPA1L:c.1478C>T were tested for Hardy-Weinberg equilibrium using the chi-square test. The genotype and allele frequencies of these polymorphisms were analysed with Fisher's exact test. Statistical significance was defined as p<0.05. The odds ratio (OR) was calculated to measure the

strength of the association observed.

Results

Of the 140 males in the study, 68(28.5%) were infertile cases with a mean age of 27.83±5.04 years and 72(51.4%) age-matched fertile controls with a mean age of 30.70±5.15 years. There were no significant differences between the groups in terms of age,, body mass index (BMI), semen volume, total sperm count (p=0.66), motility and sperm morphology (p>0.05 each) (Table-1). The genotype frequencies observed for the HSPA1B: c.1059G>A and HSPA1L:c.1478C>T polymorphisms also did not differ significantly from those expected under the Hardy-Weinberg equilibrium (p>0.05). For the HSPA1B: c.1059 polymorphism, the AA genotype and the A allele frequencies in the infertile males did not differ from those of the healthy controls (47.0% and 45.8% vs. 64.6% and 66.7%: p=0.750 vs. p=0.920, respectively).

There was no significant association between the infertile

Table-1: Demographical and sperm features (Mean ± standard deviation).

	Infertile patients (n=68)	Fertile controls (n=72)	P-value
Age (year)	27.83 ± 5.04	30.70 ± 5.15	0.570
BMI (kg/m ²)	23.93 ± 2.54	23.32 ± 2.07	0.368
Semen volume (ml)	2.69 ± 1.15	2.83 ± 1.10	0.667
Total sperm number (million)	68.50 ± 42.76	71.91 ± 34.64	0.762
Motility (%)	66.25 ± 14.12	61.54 ± 15.93	0.284
Sperm morphology structure (%)	4.37 ± 0.64	4.41 ± 0.71	0.928

BMI: Body mass index.

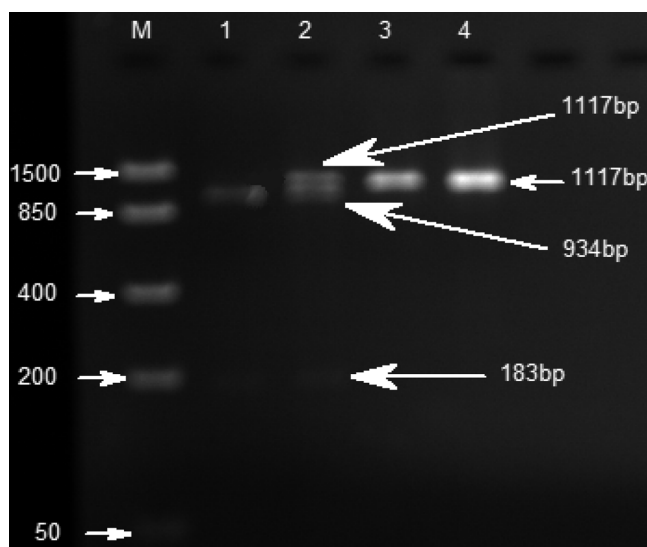


Figure-1: The restriction profile of the HSPA1B gene PstI(G>A) (c.1059G>A). Deoxyribonucleic Marker (100-1500bp, Bio Basic Inc., Canada); Lane 1: GG genotype (homozygous, wild type); lane 2: GA genotype (heterozygous); lane 3: AA genotype (homozygous, polymorphic); lane 4: undigested polymerase chain reaction product.

Table-2: Polymorphism in infertile cases and fertile controls.

SNP Genotype/Allele	Infertile male patients(n=68)	Fertile male control (n=72)	OR (95% CI)	X ²	P-value
HSPA1B:c.1059G>A					
Genotype					
GG	11 (16.2%)	9 (12.5%)	Reference	0.55	0.75
GA	25 (36.8%)	30 (41.7%)	1.460 (0.520-4.100)		
AA	32 (47.0%)	33 (45.8%)	1.260 (0.460-3.440)		
Allele					
G	47 (35.4%)	48 (33.3%)	Reference	0.08	0.92
A	89 (64.6%)	96 (66.7%)	1.050 (0.64-1.73)		
HSPA1L:c.1478C>T					
Genotype					
CC	6 (8.8%)	6 (8.4%)	Reference	0.31	0.81
CT	28 (41.2%)	33 (45.8%)	1.170 (0.340-4.06)		
TT	34 (50.0%)	33 (45.8%)	0.970 (0.270-3.31)		
Allele					
C	40 (29.4%)	45 (31.2%)	Reference	0.04	0.83
T	96 (70.6%)	99 (68.8%)	0.910 (0.550-1.520)		

SNP: Single nucleotide polymorphism. OR: Odds ratio. CI: Confidence interval. X²: Chi-Square.

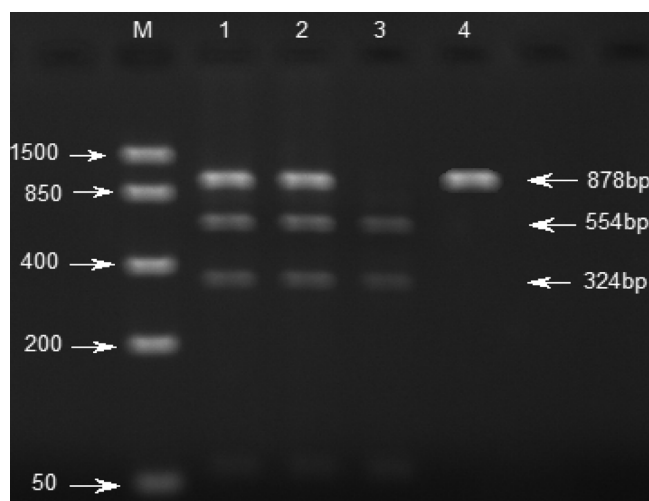


Figure-2: The restriction profile of the HSPA1L gene NcoI C>T (c.1478C>T). Lane M: Deoxyribonucleic Marker (100-1500bp, Bio Basic Inc, Canada); lane 1: CT genotype (heterozygous); lane 2: undigested polymerase chain reaction product; lane 3: TT genotype (homozygous, polymorphic); lane 4: CC genotype (homozygous, wild type).

male patients and the healthy controls in the HSPA1L.c.1478 TT genotype (50.0% vs. 45.8%, respectively) and T allele (70.6% vs. 68.8%, respectively) frequencies (Table-2).

Discussion

Male infertility is a multifactorial syndrome that accounts for about 50% of all infertilities.^{12,13} It is a heterogeneous disorder, with several genetic, environmental and behavioural factors contributing to impaired spermatogenesis.¹⁴ Despite

progress, mainly in the field of genetics, the aetiology is still unknown in about 50% cases, and the condition is termed 'idiopathic infertility'. It is currently accepted that genetics contributes to spermatogenetic failure in about 30% cases of idiopathic infertility in males.¹⁴ Over the past decades, many genetic studies have investigated the association between male infertility and genetic polymorphisms related to metabolic enzymes.^{15,16} Increasingly, the evidence suggests that polymorphisms, including the HSP70 polymorphism, in several genes are associated with male infertility.¹⁷ The HSP70 gene encodes a highly inducible stress protein, which is fundamentally important in protein transport and folding.¹⁷ Several studies have proposed that HSP loci are possible susceptibility genes in the development of many diseases, such as Grave's disease, diabetes mellitus, coeliac disease, systemic lupus erythematosus and asthma.¹⁸ Spermatogenesis is a developmental process. However, a genetic programme intrinsic to germ cells probably controls the progressive steps through mitotic, meiotic and post-meiotic phases of development. As all these developmental stages represent situations where dramatic transformations and cellular differentiation take place, it is not surprising that spermatogenesis is accompanied by the expression of different HSP.¹⁹ The participation of HSP70 is required for successful completion of meiosis during spermatogenesis. There are few studies of the relationship between the HSP70 gene polymorphism and infertility in the literature. Present studies mainly deal with HSP70-2, which is only constitutively expressed in experimental animals.²⁰⁻²² A recent study showed in a mouse model that disruption of the HSP70-2 gene using gene targeting results in failed meiosis, germ cell

apoptosis and male infertility.²⁰ Another study of HSP70 and infertility reported that the HSP70-2 protein is expressed in spermatocytes and spermatids in normal and maturation arrest tissues.²¹ However, the expression the HSP70-2 protein was low in testes with maturation arrest, and no expression of the HSP70-2 protein was demonstrated in sertoli- only specimens. Therefore, the decreased expression of the HSP70-2 protein may be associated with the pathogenesis of male infertility.²⁰ These results suggest that the HSP70-2 gene may play a specific role in human spermatogenesis and that deficiency or dysfunction of the gene may result in male infertility. HSP70 is known to be induced in several stress conditions, including heat, and has been shown to protect various human cell lines from heat-induced apoptosis.²² Many studies reported that heat shock factor is activated and that consequently HSP70 expression is increased in spermatids exposed to heat.^{23,24} One study reported that the expression of HSP70 might increase as a protective mechanism against apoptosis in the spermatozoa of infertile men.⁶ Unlike the above studies, in our study, when we compared the genotype distribution of the HSPA1B:c.1059G>A and HSPA1L:c.1478C>T gene polymorphisms between the idiopathic infertile subjects and the control subjects, we found no significant difference between the groups. One study analysed possible correlations between coding single region nucleotide polymorphisms in the HSP90 gene in patients with varicocele associated with azoospermia or severe oligozoospermia. In common with our findings, it found that the HSP90 polymorphism did not appear to be a common cause of male factor infertility.²⁵

Conclusion

Infertility in males with normal sperm parameters was not significantly associated with HSPA1B: c.1059G>A and HSPA1L:c.1478C>T gene polymorphisms. Further prospective studies with larger sample sizes and different gene groups are required to clarify this issue.

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