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HSPA1L and HSPA1B gene polymorphisms and haplotypes are associated with idiopathic male infertility in Iranian population

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ABSTRACT

Objective: Male infertility is a multifactorial disease resulting from the interaction between the genetic and environmental factors. Spermatogenic failure accounts for more than half of male infertility cases. Heat shock proteins (HSPs) are the molecular chaperones that are involved in different developmental stages of spermatogenesis. The current study was planned to investigate the role of *HSPA1L* rs2227956 and *HSPA1B* rs1061581 gene polymorphisms in idiopathic male infertility.
Study Design: This case-control study was conducted on 516 subjects consisted of 308 patients with idiopathic male infertility and 208 age matched-(±5) control subjects. *HSPA1L* rs2227956 and *HSPA1B* rs1061581 polymorphisms were genotyped by PCR-RFLP method.
Results: A significant association with male infertility was found for *HSPA1L* rs2227956 in genotypes (TT vs CT: OR = 2.049, 95% CI = 1.337–3.139, P = 0.001; TT vs CC: OR = 3.028, 95% CI = 1.100–8.332, P = 0.032). In the dominant genetic model, rs2227956C allele increased the risk of male infertility (OR = 2.049, 95% CI = 1.337–3.139, P = 0.001). Also, the results showed a significant association between the *HSPA1B* rs1061581GG genotype and male infertility (OR = 2.638, 95% CI: 1.001–4.486, P = 0.001). The rs1061581 G allele was a risk factor for male infertility (OR = 1.657, 95% CI = 1.278–2.148, P < 0.001). Haplotype analysis showed CG and TA (rs2227956/ rs1061581) haplotype affect the risk of male infertility (P < 0.001).
Conclusion: HSPA1L rs2227956 and *HSPA1B* rs1061581 gene polymorphisms are associated with susceptibility to idiopathic male infertility in Iranian population. Further studies in different ethnicity are necessary to confirm these results.

1 Introduction

Infertility is a disorder of the reproductive system characterized by the inability to gain a pregnancy following 12 months or more of regular unprotected sexual intercourse [1]. Infertility influences up to 15% of reproductive-aged couples global, with male factor being recognized in 50% of the patients [2]. Most patients of male infertility are idiopathic, apart from numerous known etiologies, including obstruction of deferent duct, sexual dysfunction, cryptorchidism and varicocele [3]. Etiological factors acting at pre-testicular, testicular, or post-testicular level may change Spermatogenesis [4]. Regulation of this process via expression of several genes is very critical because disruption of the cell cycle, apoptosis and DNA damage may result in atypical spermatogenesis [5–7].

Heat shock proteins (HSPs) are involved in the various developmental stages of spermatogenesis such as dramatic transformations and cell differentiation [8]. HSPs are expressed in response to different various factors like extreme heat, oxidative stress, hypoxia, ultraviolet light, cell damage, heavy metals, toxins, viruses, chemical poisons, and aging [9,10]. They are now known to play numerous roles, even in unstressed cells, in successful folding, assembly, intracellular localization, secretion, regulation, and degradation of other proteins [11]. The human HSPs have been classified into six families, according to their molecular weight, namely, HSPA (HSP70), HSPC (HSP90), HSPD/HSPE (HSP60/HSP10), HSPH (HSP110), DNAJ (HSP40) and HSPB (small HSP) [12]. The HSP70 family is composed of HSPA1A (HSP70-1), HSPA1B (HSP70-2) and HSPA1L (HSP70-hom) genes [13]. An inverse correlation between HSP 70 expression and sperm concentration has been reported and indicated that the increased HSP 70 expression is positively correlated to DNA damage in sperm [14,15]. Also,

Abbreviations: CI, confidence interval; DNAD, eoxyribonucleic Acid; HSP, heat shock protein; HWE, hardy–weinberg equilibrium; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SD, standard deviation.

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spermatogenesis is critically dependent on chaperones especially HSPA2, and even mild stress conditions can trigger a massive demise of spermatocytes [16–18]. In the current study, we investigated the association of *HSPA1L* rs2227956 and *HSPA1B* rs1061581 gene polymorphisms with idiopathic male infertility in Iranian population.

2 Methods and materials

2.1 Subjects

This case–control study was conducted on 516 men included 208 fertile men and 308 patient men with idiopathic infertility who were recruited from Dr Rostami infertility center in Shiraz, south of Iran from March 2015 to February 2017. Patient group had been unable to conceive for at least 12 months and underwent a complete history and physical examination. The control group consisted of fertile men who had at least one child. All of the fertile controls had normal semen parameters and were matched with patients for age. Male factor exclusion criteria included: abnormal sexual and ejaculatory functions, infection or other agents suspected to be associated with male reproduction, immune infertility, microdeletions of Y chromosome and related specific occupations (such as gasoline filling station workers and heat exposed worker). Peripheral whole blood samples were collected from subjects and used for genomic DNA extraction as described previously [19]. Informed written consent was obtained from each participant and the study was approved by the ethnic committee of Shiraz University of Medical Sciences, Shiraz, Iran.

2.2 Genotyping

HSPA1L rs2227956 and *HSPA1B* rs1061581 genotype determination was performed using polymerase chain reaction (PCR)-based Restriction Fragment Length Polymorphism (RFLP) technique with some change according to a previously described method [20]. Briefly, the fragments spanning polymorphic sites were amplified using the following primers: forward: 5′-GGACAAGTCTGAGAAGG-TACAG-3′, reverse: 5′-GTAACTTAGATTCAGGTCTGG-3′ (*HSPA1L*) and forward: 5′-CATCGACTTCTACACACGTCCA-3′, reverse: 5′-CAAAGTCCTTGAGTCCCAAC-3′ (*HSPA1B*). PCR reaction conditions were an initial denaturation at 94 °C for 5 min followed by 35 cycles with the following conditions: 94 °C for 45 s, 55 °C (*HSPA1L*) or 63 °C (*HSPA1B*) for 45 s and 72 °C for 45 s with a final extension at 72 °C for 7 min. For RFLP detection, the *HSPA1L* and *HSPA1B* PCR products were digested with *NCOI* and *PstI* respectively (Fig. 1).

2.3 Statistical analysis

Statistical analysis was done by using the SPSS 17. Hardy–Weinberg analysis was performed to compare the observed and expected genotype frequencies using $\chi 2$ test. Logistic regression analysis was used to calculate odds ratio (OR) and 95% confidence intervals (CI) of genetic susceptibility to idiopathic male infertility. Haplotype analysis was finally conducted using SHEsisPlus online software (http://analysis.bio-x.cn). Differences were considered significant when $P < 0.05$.

3 Results

3.1 Characteristics of population

The study groups consist of 308 clinically idiopathic infertile men (mean age ± SD: 35.061 ± 6.812 years) and 208 fertile men (36.744 ± 7.822 years). There was no significant difference between groups regarding age (P = 0.0823). Semen parameters of patients and controls are shown in Table 1. Evaluation of smoking status in case and control groups showed that there was no significant association (OR:1.237, 95% CI: 0.761–2.010, P = 0.391) between smoking and male infertility (data not shown).

3.2 Associations between polymorphisms and male infertility

The genotype frequencies of the control subjects in *HSPA1*B (χ^2 = 0.958, df = 1, P = 0.324) and in *HSPA1L* rs2227956 (χ^2 = 1.990, df = 1, P = 0.158) did not show significant deviation from Hardy–Weinberg equilibrium.

3.3 HSPA1L *rs2227956*

There were significant differences in the *HSPA1L* rs2227956 genotype and allele frequencies between the case and control groups. As shown in Table 2, statistical significant difference was observed in the CT (OR = 2.049, 95% CI = 1.337–3.139, P = 0.001) and CC (OR = 3.028, 95% CI = 1.100–8.332, P = 0.032) genotype distributions between patient and control groups. In addition, the C allele is associated with the increased risk of idiopathic male infertility (OR = 2.049, 95% CI = 1.337–3.139, P = 0.001).

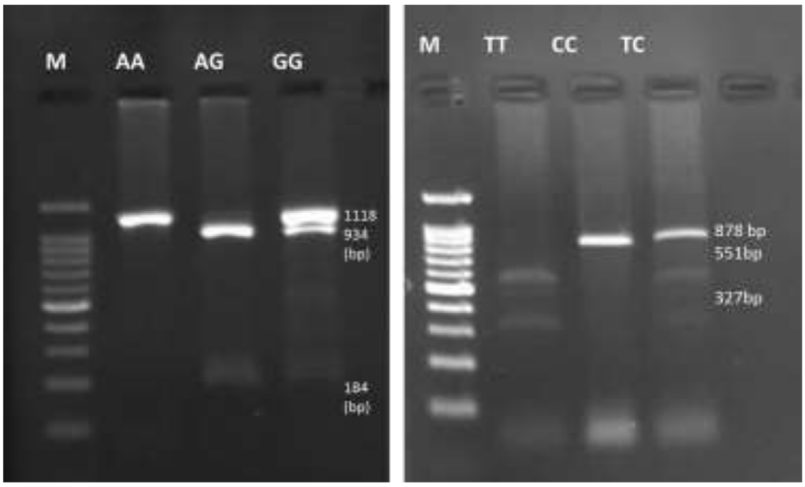

Fig. 1. Electrophoresis pattern of PCR-RFLP reaction for the detection of *HSPA1L* rs2227956 and *HSPA1B* rs1061581 polymorphisms. M: 100bp DNA ladder.

Table 1
Semene parameters of patient and control groups.

Semen parameters	Control Mean ± SD	Patient Mean ± SD	P
Semen volume	3.408 ± 1.645	3.354 ± 1.658	0.776
Sperm morphology (Normal Form %)	34.688 ± 15.260	33.290 ± 20.914	0.504
Total sperm number (10^6 per ejaculate)	130 ± 133	95.2 ± 130	0.04
Total motility (PR + NP, %)	60.2 ± 45	52.3 ± 52.7	0.39
Vitality (Live sperm, %)	61.259 ± 19.939	57.412 ± 23.082	0.36
Progressive motility(PR,%)	47.3 ± 16.8	32.6 ± 23	<0.001
Sperm concentration (10^6 per ml)	75.9 ± 44.4	45.1 ± 45.8	<0.001

Table 2
Association between *HSPA1L* rs2227956 polymorphism and idiopathic male infertility.

HSPA1L genotypes	Control (N %)	Patient (N %)	OR(95% CI)	P
Codominant				
TT	164(78.8)	195(63.3)	1	–
CT	39(18.8)	95(30.8)	2.049(1.337–3.139)	**0.001**
CC	5(2.4)	18(5.8)	3.028(1.100–8.332)	**0.032**
Dominant				
TT	164(78.8)	195(63.3)	1	–
TC + CC	44(21.2)	113(36.7)	2.160(1.440–3.240)	<**0.001**
Recessive				
TT + TC	203(97.6)	290(94.2)	1	
CC	5(2.4)	18(5.8)	2.520(0.921–6.898)	0.072
Allele				
T	367(88)	485(79)	1	–
C	49(12)	131(21)	2.023(1.418–2.886)	<**0.001**

3.4 HSPA1B *rs1061581*

The genotype and allele distribution of *HSPA1B* rs1061581 polymorphism in patients and controls are shown in Table 3. A significant difference was found between two groups with regard to this polymorphism (χ^2: 13.336, P: 0.001). The *HSPA1B* rs1061581 polymorphism was a risk factor for susceptibility to infertility in dominant (AA vs. AG + GG: OR = 1.674, 95% CI = 1.168–2.397, P = 0.005) and recessive (AA + AG vs GG: OR = 2.233, 95%CI = 1.362-–3.361, P = 0.001) tested inheritance models. Moreover, the rs1061581 G allele was shown as a risk factor for predisposition to male infertility (OR = 1.657, 95% CI = 1.278–2.148, P < 0.001).

Table 3
Association between *HSPA1B* rs1061581 polymorphism and idiopathic male infertility.

HSPA1L genotypes	Control (N%)	Patient (N%)	OR(95% CI)	P
Codominant				
AA	98(47.1)	107(34.7)	1	–
AG	85(40.9)	129(41.9)	1.390(0.944–2.048)	0.096
GG	25(12)	72(23.4)	2.638(1.551–4.486)	<0.001
Dominant				
AA	98(47.1)	107(34.7)	1	–
AG + GG	58(52.9)	201(65.3)	1.674(1.168–2.397)	0.005
Recessive				
AA + AG	183(88)	236(76.6)	1	
GG	25(12)	72(23.4)	2.233(1.362–3.361)	0.001
Allele				
A	281(67)	343(56)	1	–
G	135(33)	273(44)	1.657(1.278–2.148)	<0.001

3.5 Haplotype analysis

The results of the haplotype analysis between case and control groups are presented in Table 4. The frequency of the CG (rs2227956/rs1061581) haplotype was significantly higher in cases than in controls (OR = 3.923; 95% CI = 1.933–7.962, P < 0.001). Moreover, TA (rs2227956/rs1061581) haplotype was associated with decreased risk of male infertility (OR = 0.539; 95% CI = 0.419-0.694, P < 0.001). The differences in the frequencies of other haplotypes were not significant between case and control groups.

4 Discussion

In the current study, association between *HSPA1L* rs2227956 and *HSPA1B* rs1061581 gene polymorphisms and idiopathic male infertility have been investigated. The results confirmed that a positive association between these polymorphisms and idiopathic male infertility in Iranian population.

HSPs can modify many of the physiological functions of numerous different proteins and therefore critical for cell survival [21]. Despite the fact that the HSPs are often related to the cellular stress response, they also play an important function in supporting normal cell processes which include development and differentiation [22]. HSPs are involved in the different developmental stages of spermatogenesis such as dramatic transformations and cellular differentiation [23]. The importance of heat shock proteins for sperm development also extends to synergistic roles associated with the functional transformation of these cells that occurs during their successive phases of post-testicular maturation within the epididymal maturation of male and capacitation of female reproductive tracts [24]. Among the numerous families of HSP that have been implicated in the regulation of reproductive system development and function, HSP70 have emerged as being indispensable for male fertility [25]. Decreased expression of the HSPA2 gene, has been reported to be associated with the pathogenesis of male infertility [26]. Erata et al. found a positive correlation between the expression of HSP70 and sperm DNA damage in male infertility. It seems to confirm that increased HSP70 expression would assist in blocking aggregation and refolding of damaged proteins as a general protective response [27].

Among the HSP70 chaperone members, HSPA1B and HSPA1L are two testis specific isoforms that are mainly expressed in the mammalian testis and sperm. They are regulated developmentally and expressed specifically in spermatogenic cells [28,29]. The expression of *HSPA1L* peaks in spermatides and is not influenced by heat [30]. Bohring & Krause (2003) have found the two HSP70 family members HSPA2 and HSPA1L were among the human sperm membrane antigens recognized by antisperm antibodies from seminal plasma samples of infertile men [31]. Also, a recent study showed in a mouse model that disruption of the *HSP70-2* (*HSPA1B*) gene results in failed meiosis, germ cell apoptosis and male infertility [32]. Therefore, it seems that the decreased expression or activity of the HSPA1B and HSPA1L proteins may be associated with the pathogenesis of male infertility.

Table 4
Haplotype association of *HSPA1L* rs2227956 and *HSPA1B* rs1061581 variants with male infertility risk.

HSPA1L rs2227956	*HSPA1B* rs1061581	Case (frequency)	Control (frequency)	OR (95% CI)	P
C	A	0.130	0.095	1.415 (0.946–2.118)	0.090
C	G	0.083	0.023	3.923 (1.9337.962)	<0.001
T	A	0.427	0.580	0.539 (0.4190.694)	<0.001
T	G	0.360	0.302	1.301 (0.9971.698)	0.052

The *HSPA1L* rs2227956 or +2437 T/C polymorphism results in a Met to Thr amino acid replacement at position 493 in the peptide-binding domain affecting the substrate specificity and chaperone activity of the HSPA1L protein [33]. *HSPA1B* rs1061581 or 1267A/G polymorphism is due to an A–G substitution in the coding sequence of the *HSPA1B*. It has been reported that the G allele leads to reduced expression of the *HSPA1B* and it can be inferred that the subjects with the GG genotype are at higher risk toward improper folding of the proteins due to transcriptional insufficiency [34].

In the present study, we showed that *HSPA1L* rs2227956 C allele and *HSPA1B* G allele are associated with increased risk of male infertility. In previous studies, the association of HSP70 polymorphisms and some diseases such as Long QT syndrome [34], Idiopathic pulmonary fibrosis [35] and systemic lupus Erythematosus [36] were investigated. Recently, the association between *HSPA1L* rs2227956 and *HSPA1B* rs1061581 gene polymorphisms and male infertility was studied in Turkish population; the results showed that infertility in males with normal sperm parameters was not significantly associated with these gene polymorphisms [37]. Unlike the results of this research, our results in a larger population study, showed that the rs2227956 and rs1061581 polymorphisms increased the risk of idiopathic male infertility in Iranian population. The reasons for these discrepancies are unclear, but may have been caused by ethnic heterogeneity and criteria for recruitment.

In conclusion, the present population-based case-control study indicated that both *HSPA1L* rs2227956 and *HSPA1B* rs1061581 gene polymorphisms affect the risk of male infertility in Iranian population.

Declaration of competing interest

None declared.

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References

[1] Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem 2018;1(18) pii: S0009-912030220-0.
[2] Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. Fertil Steril 2002;77(5):873–82.
[3] Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. EAU guidelines on male infertility. Eur Urol 2005;48(5):703–11.
[4] Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European association of urology guidelines on male infertility: the 2012 update. Eur Urol 2012;62(2):324–32.
[5] Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update 2003;9(4):331–45.
[6] Holstein AF, Schulze W, Davidoff M. Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol 2003;1(1):107.
[7] Zini A, Blumenfeld A, Libman J, Willis J. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. Hum Reprod 2005;20(4):1018–21.
[8] Meinhardt A, Wilhelm B, Seitz J. Mini symposium. New aspects of spermatogenesis. Expression of mitochondrial marker proteins during spermatogenesis. Hum Reprod Update 1999;5(2):108–19.
[9] Chen B, Feder ME, Kang L. Evolution of heat-shock protein expression underlying adaptive responses to environmental stress. Mol Ecol 2018;27(15):3040–54.
[10] Wang Y, Zhou F, Wu Y, Xu D, Li W, Liang S. The relationship between three heat shock protein 70 gene polymorphisms and susceptibility to lung cancer. Clin Chem Lab Med 2010;48(11):1657–63.
[11] Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP. Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals (Basel) 2017;11(1).
[12] Jee H. Size dependent classification of heat shock proteins: a mini-review. J Exerc Rehabil 2016;12(4):255–9.
[13] Zuiderweg ER, Hightower LE, Gestwicki JE. The remarkable multivalency of the Hsp70 chaperones. Cell Stress Chaperones 2017;22(2):173–89.
[14] Sisti G, Kanninen TT, Ramer I, Witkin SS. Interaction between the inducible 70-kDa heat shock protein and autophagy: effects on fertility and pregnancy. Cell Stress Chaperones 2015;20(5):753–8.
[15] Erata GÖ, Toker NK, Durlanık Ö, Kadıoğlu A, Aktan G, Toker GA. The role of heat shock protein 70 (Hsp 70) in male infertility: is it a line of defense against sperm DNA fragmentation? Fertil Steril 2008;90(2):322–7.
[16] Rockett JC, Mapp FL, Garges JB, Luft JC, Mori C, Dix DJ. Effects of hyperthermia on spermatogenesis, apoptosis, gene expression, and fertility in adult male mice. Biol Reprod 2001;65(1):229–39.
[17] Nixon B, Bromfield EG, Dun MD, Redgrove KA, McLaughlin EA, Aitken RJ. The role of the molecular chaperone heat shock protein A2 (HSPA2) in regulating human sperm-egg recognition. Asian J Androl 2015;17(4):568.
[18] Paul C, Teng S, Saunders PT. A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. Biol Reprod 2009;80(5):913–9.
[19] Hosseini AH, Kohan L, Aledavood A, Rostami S. Association of miR-146a rs2910164 and miR-222 rs2858060 polymorphisms with the risk of polycystic ovary syndrome in Iranian women: a case–control study. Taiwan J Obstet Gynecol 2017;56(5):652–6.
[20] Medhi S, Sarma MP, Asim M, Kar P. G enetic variants of heat shock protein A1L2437 and A1B1267 as possible risk factors for hepatocellular carcinoma in India. J Viral Hepat 2013;20(4):e141–7.
[21] Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Ulrich Hartl F. Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 2013;82:323–55.
[22] Åkerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 2010;11(8):545.
[23] Dun MD, Aitken RJ, Nixon B. The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Hum Reprod Update 2012;18(4):420–35.
[24] Dun MD, Aitken RJ, Nixon B. The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Hum Reprod Update 2012;18(4):420–35.
[25] Nixon B, Bromfield EG, Cui J, De Iuliis GN. Heat shock protein A2 (HSPA2): regulatory roles in germ cell development and sperm function. Adv Anat Embryol Cell Biol 2017;222:67–93.
[26] Ergur AR, Dokras A, Giraldo JL, Habana A, Kovanci E, Huszar G. Sperm maturity and treatment choice of in vitro fertilization (IVF) or intracytoplasmic sperm injection: diminished sperm HspA2 chaperone levels predict IVF failure. Fertil Steril 2002;77(5):910–8.
[27] Erata GÖ, Toker NK, Durlanık Ö, Kadıoğlu A, Aktan G, Toker GA. The role of heat shock protein 70 (Hsp 70) in male infertility: is it a line of defense against sperm DNA fragmentation? Fertil Steril 2008;90(2):322–7.
[28] Naaby-Hansen S, Herr JC. Heat shock proteins on the human sperm surface. J Reprod Immunol 2010;84(1):32–40.
[29] Allen JW, Dix DJ, Collins BW, Merrick BA, He C, Selkirk JK, et al. HSP70-2 is part of the synaptonemal complex in mouse and hamster spermatocytes. Chromosoma 1996;104(6):414–21.
[30] Ito Y, Ando A, Ando H, Ando J, Saijoh Y, Inoko H, et al. Genomic structure of the spermatidspecific hsp70 homolog gene located in the class III region of the major histocompatibility complex of mouse and man. J Biochem 1998;124:347–53.
[31] Bohring C, Krause W. Characterization of spermatozoa surface antigens by antisperm antibodies and its influence on acrosomal exocytosis. Am J Reprod Immunol 2003;50:411–9.

[32] Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, et al. Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci U S A. 1996;93(8):3264–8.
[33] Milner CM, Campbell RD. Polymorphic analysis of the three MHC-linked HSP70 genes. Immunogenetics 1992;36(6):357–62.
[34] Ali A, Qureshi SF, Medikare V, Venkateshwari A, Calambur N, Rao H, et al. Heat shock protein 70 gene polymorphisms' influence on the electrophysiology of long QT syndrome. J Interv Card Electrophysiol 2016;45(2):119–30.
[35] Aquino-Gálvez A, González-Ávila G, Pérez-Rodríguez M, Partida-Rodríguez O, Nieves-Ramírez M, Piña-Ramírez I, et al. Analysis of heat shock protein 70 gene polymorphisms Mexican patients with idiopathic pulmonary fibrosis. BMC Pulm Med 2015;15(1):129.
[36] Fürnrohr BG, Wach S, Kelly JA, Haslbeck M, Weber CK, Stach CM, et al. Polymorphisms in the Hsp70 gene locus are genetically associated with systemic lupus erythematosus. Ann Rheum Dis 2010;69(11):1983–9.
[37] Ciftci H, Celepkolo B, Dilmec F, Koksal M, Yeni E, Yagmur I, et al. Genetic polymorphisms of hspa1b and hspa1l in infertile men. J Pak Med Assoc 2015;65(7):701–4.