Am J Clin Exp Immunol 2024;13(6):278-284
www.ajcei.us /ISSN:2164-7712/AJCEI0161327

Original Article

Heat shock protein 70 gene polymorphisms in Iranian patients with Multiple sclerosis

Azam Bakhshandeh, Alireza Kargar Dolatabadi, Touraj Farazmanfar, Majid Shahbazi

Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Received October 20, 2024; Accepted December 11, 2024; Epub December 25, 2024; Published December 30, 2024

Abstract: Genetic factors are effective reagents in susceptibility to multiple sclerosis (MS). Previous studies have shown the relationship between heat shock protein (HSP) gene polymorphisms. So, HSP70 single nucleotide polymorphisms (SNPs) were evaluated as MS risk factors. Here, DNA genotyping was done for HSP70 gene polymorphisms, including HSP70-1 +190 G>C, HSP70-1 -110 A>C, HSP70-1 +438 A>C, and HSP70-hom +2437 A>G in two groups including Iranian MS patients and controls. A standard phenol/chloroform method isolated DNA samples from peripheral blood. Sequence-specific amplification (SSP) polymerase chain reaction (PCR) was used for genotyping polymorphisms. Overall, 76 (35.80%) MS patients and 136 (65.10%) controls were studied with an age mean of 36.0 ± 8.0 years. Female/male was significantly higher in patients than in controls (4.43 vs. 0.10, $P < 0.001$). The average age was significantly lower in patients ($P < 0.001$). The most common clinical feature was relapsing-remitting (RR) MS; more than half of the population was Fars. Results showed that genotypes of HSP70-hom +2437 C>T had a significant relation with MS (OR = 2.0, 95% CI = 1.0-5.0, P = 0.03) and the same applies to HSP70-1 -110 A>C (OR = 0.0, 95% CI = 0.0-1.0, P < 0.001). Allele and genotype frequency of two other HSP70 SNPs (HSP70-1 +190 G>C, HSP70-1 +438 A>C) showed no significant differences between patients and controls. HSP70-hom +2437 C>T and HSP70-1 -110 A>C can be considered as risk factors for MS in our population. However, other HSP SNPs should be studied in a larger population in the future.

Keywords: HSP70-1, DNA genotyping, relapsing-remitting multiple sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease with inflammation and myelin sheath damage in the central nervous system [1]. Globally, the prevalence of the disease is about 2.5 million people [2]. Iran is a medium prevalence country (with 5 to 30 MS cases per 100000 population) [3]. The evidence reported a higher prevalence of MS in the northern hemisphere than in the southern [4]. The most common age of this disease is 20-40 years old, and its prevalence in women is twice that of men [5]. The main reason for this disease has yet to be determined. A series of risk factors affect the disease and its occurrences, such as immune system failure, genetic predisposition, family background, geographic region, viral infections, and psychological pressures [6]. Like other chronic inflammatory disorders, manifestations of MS are various, from benign disease to rapidly developing and debilitating disease. MS may affect many organs and systems of the body and causes disability, reduction of the life quality of patients, and death [7, 8]. Sclerotic plaques are the essential symptom of MS that occurs in an inflammatory process, demyelination, and repair with the loss of different axons [9].

Genetic and environmental factors are effective reagents in MS. HSPs are a group of proteins expressed under stress conditions, such as free radicals [10]. As oxidative stresses affect MS pathogenesis and progression, HSPs are involved in this disease [11]. HSPs act as housekeeping genes in physiological conditions and as molecular chaperones. These proteins play roles in the suitable folding of newly synthesized proteins; the functions of HSPs are essential in preventing protein accumulation, weakening unstable and incompatible proteins, and transferring proteins in cell parts [12-16]. High expression of HSPs in the second autoinduc-

https://doi.org/10.62347/CMYA9839

tion, such as heat shock, promotes the development of cell survival function [15]. These proteins consist of HSP100, HSP90, HSP70, HSP60, and HSP40 families and small families [17]. According to previous studies, HSP plays sone roles in neurodegenerative diseases. Especially, HSP70 expression has been observed in CNS that is a powerful antiapoptotic protein by inhibition of programmed cell death signaling. Functions of HSP70 are as intracellular (acting as a chaperone protein) and extracellular (triggering immunological responses) models. The functions of HSP70 in MS pathogenesis are complex. Some evidences reported HSP70 is overexpressed in the CNS of MS patients that is related to the neuroprotective function of HSP70 in an inflammatory environment. On the other hand, high quantities of HSP70 are released into the milieu, when overexpression of HSP70 fails to prevent cell death and promote the activation of immune system mediated by its cytokinelike property [18].

The cytoprotective and immunomodulatory functions of HSP70 can be affected by different polymorphisms of it that cause quantitative and qualitative changes in HSP70 expression [11]. Some studies have shown the relationship between HSP70 gene polymorphisms and different polymorphisms studied in MS, including HSP70-2 1267 A/G, HSP70-hom 2437 T/C, promotor region, and 190 G/C polymorphism of HSP70-1 [17].

This study survived the relationship between four HSP70 SNP gene polymorphisms and MS using the polymerase chain reaction (PCR) method.

Materials and methods

Patients

Iranian patients with the diagnosis of MS from Golestan and Tehran provinces, Iran, were recruited in this study. A neurologist precisely diagnosed MS. The Control group included healthy blood donor volunteers from Blood Donation Centers of Gorgan and Gonbad, in the north of Iran, aged 23-62 years. Age, sex, and ethnicity matched control subjects who were randomly selected and had no autoimmune or inflammatory diseases. A demographic questionnaire was prepared for MS patients. The demographic data included sex, age, onset, and ethnicity. Informed Consent forms were completed and signed by all individuals. The exclusion criteria were lack of participant satisfaction or underlying disease. The ethics committee of Golestan University of Medical Sciences, Gorgan, Iran (IR.GOUMS.REC.1397.196) approved this study. All individuals completed and signed informed consent forms.

DNA isolation

Peripheral blood samples were collected in 5-mL coated tubes from MS patients and controls. DNA was isolated by a standard phenol/chloroform method [19] with some modifications. Red blood cells were briefly lysed three times with a buffer (ammonium chloride, potassium dihydrogen phosphate, and disodium hydrogen phosphates). The pellets were resuspended with SDS (10%) (Merck, Germany), EDTA (Merck, Germany), and 10 µL proteinase K (QIAGEN, Germany) and incubated for one hour at 65°C. After that, the samples were mixed with phenol/chloroform/isoamyl alcohol and centrifuged. The supernatants were transferred to the new tubes, and isopropanol and sodium acetate were added to visualize and precipitate the DNA. Isolated DNA were aliquoted in distilled water, and the concentration and purity of DNA were determined by spectrophotometric analysis at 260 nm (Techne, UK). All samples were stored at -80°C for future analysis.

DNA genotyping

Four HSP70 gene polymorphisms, including HSP70-1 +190 G>C (rs1043618), HSP70-1 -110 A>C (rs1008438), HSP70-1 +438 A>C, and HSP70-hom +2437 A>G (rs2227956) were genotyped by SSP PCR. The human growth hormone (HGH) gene was used as an internal control. The primers are presented in **Table 2**. Each reaction was performed with 15 µl reaction mix including 100 ng of genomic DNA, 9.5 µL master mix containing 20 ml dNTP, 1X ready-load PCR buffer, 12% sucrose (Merck, Germany), one-unit Taq polymerase (QIAGEN, Germany), and 30 µM of each specific primer (MWG, Germany). The following amplification protocol was applied in a Thermal Cycler (Techne, UK): [HSP70-1 +190 (1 min at 95°C; 10 cycles of 15 s at 95°C, 50 s at 66°C, and 40 s at 72°C; 20 cycles of 20 s at 95°C, 50 s at 60°C, and 50 s at 72°C; and 5 min at 72°C as final extension)],

Table 1. Primers used for genotyping HSP70-1 (+190 G>C, -110 C>A, +438 A>C) and HSP70-hom +2437 C>T gene polymorphisms

Polymorphism	Primer	Size (bp)
HSP70-1 +190 G>C	G: 5'-CTG CTC TCT GTC GGC TCC-3'	
	C: 5'-CTG CTC TCT GTC GGC TCG-3'	
	Generic: 5'-GCT GCG ACA GTC CAC TAC C-3'	185
HSP70-1 -110 A>C	C: 5'-CAG GAC GGG AGG CGA AAC-3'	
	A: 5'-CAG GAC GGG AGG CGA AAA-3'	
	Generic: 5'-CTC GAA AAA GGT AGT GGA CTG-3'	177
HSP70-1 +438 A>C	A: 5'-GGT CGC CGA ACT TGC GGC CA-3'	
	C: 5'-GGT CGC CGA ACT TGC GGC CG-3'	337
	Generic: 5'-CCG GCGT CCG GAA GGA CC-3'	
HSP70-hom +2437 C>T	C: 5'-TTG CCG GTG CTC TTG TCC G-3'	
	T: 5'-CTT GCC GGT GCT CTT GTC CA-3'	124
	Generic: 5'-TGG GGC GGT TTG ATC TGA C-3'	

Table 2. Demographic characteristics of patients with MS and controls

Characteristics		Patients n = 76	Controls n = 136	*P*-Value
Sex, n (%)	Male	14 (18.4)	123 (90.4)	< 0.001**
	Female	62 (81.6)	13 (9.6)	
	Female/Male	4.43	0.10	
Age (years), Mean ± SD		31.00 ± 9.00	38.00 ± 6.00	< 0.001**
Age at onset (years), Mean ± SD		25.00 ± 7.00	-	
Ethnicity, n (%)	Fars	74 (97.4)	123 (90.4)	NS
	Turkman	2 (2.6)	13 (9.5)	
Clinical feature				
Subtype, n (%)	Relapsing-remitting (RR)	55 (72.4)	-	
	Chronic-progressive (CP)	1 (1.3)	-	
	Secondary-progressive (SP)	1 (1.3)	-	
	Chronic-relapsing (CR)	11 (14.5)	-	
	Missing	8 (10.5)	-	-
Disease duration (year)	-	5.0 ± 4.1	-	-

**High significance differences (P < 0.01), NS: non significance differences (P > 0.05).

[HSP70-1 -110 (1 min at 95°C; 10 cycles of 15 s at 95°C, 50 s at 64°C, and 40 s at 72°C; 20 cycles of 20 s at 95°C, 50 s at 58°C, and 50 s at 72°C; and 5 min at 72°C as final extension)], [HSP70-1 +438 (1 min at 95°C; 10 cycles of 15 s at 95°C, 50 s at 68°C, and 40 s at 72°C; 20 cycles of 20 s at 95°C, 50 s at 53°C, and 50 s at 72°C; and 5 min at 72°C as final extension)] and [HSP70-hom +2437 (1 min at 95°C; 10 cycles of 15 s at 95°C, 50 s at 64°C, and 40 s at 72°C; 20 cycles of 20 s at 95°C, 50 s at 58°C, and 50 s at 72°C; and 5 min at 72°C as final extension)]. Primers is shown in **Table 1**. The PCR products were electrophoresed in 1.5% agarose gel (Merck, Germany). The bands were visualized on the UV transilluminator and photographed with a gel documentation system (UVITEC, UK).

Statistical analysis

Statistical analyses were performed using SPSS Version 21. The Kolmogorov-Smirnov test was used to determine the normality of data. Chi-square analyzed demographic data, and a T-test was used to compare the mean age between two patient and control groups. Allele and genotype frequencies were calculated and compared between groups by Chi-square or Fisher's exact tests. Odds ratios and 95% confi-

dence intervals were determined using logistic regression analysis. The significant level was set to $P < 0.05$.

Results

Demographic analysis

In this case-control study, 212 persons were evaluated, including 76 (35.8%) patients with MS and 136 (65.1%) controls. The average age was 36.0 ± 8.0 years old. The demographic data are presented in **Table 2**. According to the results, the ratio of females to males was significantly higher in MS patients ($P < 0.001$). However, the controls' average age was significantly higher ($P < 0.001$). The central portion of persons in both groups was Fars, and 72.4% of patients were in Relapsing-remitting (RR) status. Eighty-one percent (45 persons) of RR-MS patients and 81% (9 persons) of Chronic-relapsing multiple sclerosis (CR-MS) patients were significantly female ($P < 0.001$).

Allele and genotype analysis

Table 3 shows the allele and frequencies of *HSP70* gene polymorphisms. Results showed that genotypes of HSP70-hom +2437 C>T and HSP70-1 -110 C>A had a significant relation with MS and increased the risk of disease ($P = 0.03$ and $P < 0.001$, respectively). Genotype frequencies of HSP70-hom +2437 C>T were reported in 72 patients and 135 controls and they were 54 in patients and 102 in controls for HSP70-1 -110 C>A. HSP70-1 +190 G>C and HSP70-1 +438 A>C did not show a significant impact on MS probability ($P>0.05$).

Discussion

In this study, the information on demographic characterization was remarkable. The ratio of females to males in MS patients was 4.43, and it was 0.10 in controls. There was a significant relationship between gender and MS risk ($P < 0.001$). This is consistent with previous studies that demonstrate the ratio of women to men with MS has increased (2.3-3-5) due to changes in environment or nutrition (e.g., obesity, smoking, sun exposure, and infections) [20]. In our study, this ratio is higher than the reported ratio in other studies. The mean age was lower in MS patients significantly ($P < 0.001$). Age at onset of MS was 25.00 ± 7.00 years in our population. Our finding aligns with previous studies on the onset of MS at 20-30 years [21]. The results showed that the most common manifestation of MS was RR, which was observed in women mainly. These results are reported in existent data from other studies [22]. Most participants (patients and controls) were Fars, and ethnicity was not a risk factor for MS in this study.

Although, Tarzjani, et al., 2019, found no relationship between HSP70 polymorphism and susceptibility to MS in the Iranian population, but the results of our study are different. Noticeably, Tarzajani et al. investigated the relationship in a small sample size (n = 50) and included RR-MS patients. They studied the 1053 G>A (rs1061581) polymorphism [10]. According to genetic results, HSP70-hom +2437 T>C had a significant relation with MS, and it can directly increase the risk of disease (Adjusted OR = 2.0, 95% CI = 1.0-5.0, $P = 0.03$). HSP70-hom +2437 T>C is a member of the HSP70-hom gene family with two members that are not heat-inducible. Another member of this family is HSP70-hom +2763 G>A. The associations between both of them and susceptibility to some autoimmune diseases were recorded in previous studies. Studies showed a correlation between HSP70-hom +2437 T>C and the risk of insulin-dependent diabetes mellitus in Dutch patients [23]. Niino et al., 2001, demonstrated there were no significant differences between Japanese MS patients (n = 107) and controls (n = 82) in HSP70 SNPs, including HSP70-1 +190 G>C, HSP70-2 +1267 A>G and HSP70-hom +2437 T>C. The mean age of onset for patients was 26.9 ± 9.5 years which was close to our results. RR-MS and SP-MS were observed 71 (66.4%) and 36 (33.6%) patients, respectively (17). Also, Wang et al., 2015, demonstrated no significant relationship between HSP70-hom +2437 T>C with Graves' disease (GD) risk. They evaluated 153 chines patients with average age 27.6 ± 5.4 years [24]. The significant association between HSP70-hom +2437 T/C polymorphism with type 2 diabetes mellitus (T2DM) was confirmed in a study by Moniruzzaman et al., 2019, in the Bangladeshi population. This case-control study included 216 patients (mean age 50.38 ± 12.35 years) and 126 healthy controls [25]. According previous reports, HSP70-hom +2437 T/C polymorphism just showed associa-

Table 3. HSP70-1 (+190 G>C, -110 A>C, +438 A>C) and HSP70-hom +2437 T>C genotype and allele frequencies in MS patients and healthy controls

Allele/Genotypes	Patients n (%)	Controls n (%)	Adjusted OR (95% CI)	*P*-Value
HSP70-1 +190 G>C				
Allele				
G	81 (54.00)	169 (63.53)		
C	69 (46.00)	97 (36.47)		
Genotype			1.00 (0.0-2.00)	NS
GC	21 (27.63)	60 (44.12)		
CC	39 (51.31)	49 (30.03)		
Missing	15 (19.74)	24 (17.65)		
	1 (1.31)	3 (2.20)		
HSP70-1 -110 A>C				
Allele				
A	71 (51.45)	119 (46.48)		
C	67 (48.55)	137 (53.51)		
Genotype			0.0 (0.00-1.00)	< 0.001**
AA	15 (19.74)	26 (19.12)		
AC	41 (53.95)	67 (49.26)		
CC	13 (17.10)	35 (24.73)		
Missing	7 (4.67)	8 (5.88)		
HSP70-1 +438 A>C				
Allele				
A	22 (15.94)	54 (20.30)		
C	116 (84.06)	212 (79.70)		
Genotype			1.0 (0.0-3.0)	NS
AA	0 (0)	0 (0)		
AC	22 (28.95)	54 (71.05)		
CC	47 (61.84)	79 (58.09)		
Missing	7 (9.21)	3 (2.20)		
HSP70-hom +2437 C>T				
Allele				
C	21 (14.38)	25 (9.26)		
T	125 (85.61)	245 (90.74)		
Genotype			2.0 (1.0-5.0)	0.03*
CC	1 (1.31)	0 (0)		
CT	19 (25.00)	25 (18.38)		
TT	53 (69.74)	110 (80.88)		
Missing	3 (3.95)	1 (0.73)		

**High significance differences (P < 0.001), *Significance differences (P < 0.05), NS: non-significance differences (P > 0.05).

tion with diabetes and the relationship was not observed in MS and GD diseases. Our result is especially remarkable in comparison with Niino et al., 2001. Probably, different sample size and subtypes of MS are two reasons of the event.

Allele and genotype frequency of HSP70-1 +190 G>C and HSP70-1 +438 A>C showed no significant differences between patients and controls. However, the +190 GC genotype and +190 C allele frequencies were higher in patients than in controls. It can be important in future studies and the larger population. The impact of this SNP is on translation efficiency or post-transcriptional regulation of the *HSP701A* gene. So, the HSP70-1A protein synthesis level is lower for the +190 C allele than

the wild-type allele, +190 G [26]. Consistent with our results, Wang et al., 2015, found no significant differences in the allelic frequency of HSP70-1 +190 between GD patients and control groups [24]. There are few studies about the frequency of HSP70-1 +438 A>C in patients. Spagnolo et al., 2007, showed a significant increase in the HSP70-1 +438 C allele frequency in patients with sarcoidosis [27]. Here, HSP70-1 -110 A>C had a powerful effect on MS susceptibility ($P < 0.001$) and, clearly, allele A showed a relationship with MS. Also, Ghanayem et al., 2015, reported a significant association between HSP70-1 -110 A/C and renal complications in egyptioan patients with T2DM. They evaluated 60 diabetic patients and 20 healthy individuals [28].

In Conclusion, the age, gender, and clinical features of patients were similar to those of other studies. HSP70-hom +2437 C>T and HSP70-1 -110 A>C can be considered as risk factors for MS in our population. Although other HSP SNPs showed no significant relation with MS susceptibility here, the association between these gene polymorphisms can be substantial in a larger population in the future.

Acknowledgements

We thank the Golestan Blood Donor Center and all study Participants.

Disclosure of conflict of interest

None.

Address correspondence to: Majid Shahbazi, Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Shastkola Road, Falsafi Campus, Gorgan 4934174516, Iran. Tel: +98-1732451663; Fax: +98-1732451657; E-mail: shahbazim@goums.ac.ir

References

[1] Matthews WB and Compston A. Multiple sclerosis. London: Longman; 1991.

[2] Shepard CJ, Cline SG, Hinds D, Jahanbakhsh S and Prokop JW. Breakdown of multiple sclerosis genetics to identify an integrated disease network and potential variant mechanisms. Physiol Genomics 2019; 51: 562-577.

[3] Cheraghmakani H, Baghbanian SM, Habibi-Saravi R, Azar A and Ghasemihamedani F. Age and sex-adjusted incidence and yearly prevalence of multiple sclerosis (MS) in Mazandaran province, Iran: an 11-years study. PLoS One 2020; 15: e0235562.

[4] Asouri M, Alinejad Rokni H, Sahraian MA, Fattahi S, Motamed N, Doosti R, Amirbozorgi G, Karimpoor M, Mahboudi F and Akhavan-Niaki H. Association of HLA-DRA and IL2RA polymorphisms with the severity and relapses rate of multiple sclerosis in an Iranian population. Rep Biochem Mol Biol 2020; 9: 129-139.

[5] Dutta R and Trapp BD. Pathogenesis of axonal and neuronal damage in multiple sclerosis. Neurology 2007; 68 Suppl 3: 22-31; discussion S43-54.

[6] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33: 1444-52.

[7] Runmarker B and Andersen O. Prognostic factors in a multiple sclerosis incidence cohort with twenty-five years of follow-up. Brain 1993; 116: 117-134.

[8] Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J and Ebers GC. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989; 112: 133-146.

[9] Compston A and Coles A. Multiple sclerosis. Lancet 2008; 372: 1502-1517.

[10] Chavoshi Tarzjani SP, Shahzadeh Fazeli SAH, Sanati MH and Nabavi SM. Heat shock protein 70 and the risk of multiple sclerosis in the iranian population. Cell J 2019; 20: 599-603.

[11] Boiocchi C, Monti MC, Osera C, Mallucci G, Pistono C, Ferraro OE, Nosari G, Romani A, Cuccia M, Govoni S, Pascale A, Montomoli C and Bergamaschi R. Heat shock protein 70-hom gene polymorphism and protein expression in multiple sclerosis. J Neuroimmunol 2016; 298: 189-193.

[12] Flynn GC, Chappell TG and Rothman JE. Peptide binding and release by proteins implicated as catalysts of protein assembly. Science 1989; 245: 385-90.

[13] Beckmann RP, Mizzen LE and Welch WJ. Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science 1990; 248: 850-4.

[14] Hartl FU and Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 2002; 295: 1852-1858.

[15] Murakami H, Pain D and Blobel G. 70-kD heat shock-related protein is one of at least two distinct cytosolic factors stimulating protein import into mitochondria. J Cell Biol 1988; 107:2051-2057.

[16] Shi Y and Thomas JO. The transport of proteins into the nucleus requires the 70-kilodalton

heat shock protein or its cytosolic cognate. Mol Cell Biol 1992; 12: 2186-92.
[17] Niino M, Kikuchi S, Fukazawa T, Yabe I, Sasaki H and Tashiro K. Heat shock protein 70 gene polymorphism in Japanese patients with multiple sclerosis. Tissue Antigens 2001; 58: 93-96.
[18] Mansilla MJ, Montalban X and Espejo C. Heat shock protein 70: roles in multiple sclerosis. Mol Med 2012; 18: 1018-1028.
[19] Shahbazi M, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC, Hutchinson PE, Osborne JE, Lear JT, Smith AG and Hutchinson IV. Association between functional polymorphism in EGF gene and malignant melanoma. Lancet 2002; 359: 397-401.
[20] Harbo HF, Gold R and Tintoré M. Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord 2013; 6: 237-248.
[21] Prosperini L, Lucchini M, Ruggieri S, Tortorella C, Haggiag S, Mirabella M, Pozzilli C and Gasperini C. Shift of multiple sclerosis onset towards older age. J Neurol Neurosurg Psychiatry 2022; 93: 1137-1139.
[22] Romero-Pinel L, Bau L, Matas E, León I, Muñoz-Vendrell A, Arroyo P, Masuet-Aumatell C, Martínez-Yélamos A and Martínez-Yélamos S. The age at onset of relapsing-remitting multiple sclerosis has increased over the last five decades. Mult Scler Relat Disord 2022; 68: 104103.
[23] Bogunia-Kubik K, Koscinska K, Suchnicki K and Lange A. HSP70-hom gene single nucleotide (+2763 G/A and +2437 C/T) polymorphisms in sarcoidosis. Int J Immunogenet 2006; 33: 135-140.
[24] Wang YP, Tang Z, Peng BK, Zhen Q, Zhou SL and Jin XF. Heat shock protein 70 polymorphisms in Chinese patients with Graves' disease. Genet Mol Res 2015; 14: 18376-18383.
[25] Moniruzzaman M, Ahmed I, Huq S, All Mahmud MS, Begum S, Mahzabin Amin US, Rahman MH, Sarker PK, Hossain MU, Das KC and Salimullah M. Association of polymorphism in heat shock protein 70 genes with type 2 diabetes in Bangladeshi population. Mol Genet Genomic Med 2020; 8: e1073.
[26] Kowalczyk M, Owczarek A, Suchanek R, Paul-Samojedny M, Fila-Danilow A, Borkowska P, Kucia K and Kowalski J. Heat shock protein 70 gene polymorphisms are associated with paranoid schizophrenia in the Polish population. Cell Stress Chaperones 2014; 19: 205-215.
[27] Spagnolo P, Sato H, Marshall SE, Antoniou KM, Ahmad T, Wells AU, Ahad MA, Lightman S, du Bois RM and Welsh KI. Association between heat shock protein 70/Hom genetic polymorphisms and uveitis in patients with sarcoidosis. Invest Ophthalmol Vis Sci 2007; 48: 3019-3025.
[28] Ghanayem NM, El-Shafie MK, Badr EA, Elnour E, Khamis SS and Abd El Gayed EM. Study of the heat shock protein 70-1 gene polymorphism and the risk of nephropathy in type II diabetic patients. MMJ 2014; 27: 582.