Serum levels of free heat shock protein 70 and anti-HSP70 are elevated in Behçet's disease

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ABSTRACT

Objectives. *As heat shock proteins (HSPs) are described as candidate self-antigens in Behçet's disease (BD), free HSP70 and anti-HSP70 levels were measured in the sera of patients to investigate their role in the pathogenesis of BD.*

Methods. *Free human HSP70 levels were measured in the sera of patients with BD and compared to disease controls [patients with rheumatoid arthritis (RA) or recurrent oral ulcerations (ROU)] and healthy controls (HC) using ELISA. Anti-HSP70 antibody levels were also determined.*

Results. *Free human HSP70 levels were significantly elevated in BD sera (1.12±0.86 ng/ml) compared to HC (0.67±0.46 ng/ml, BD vs. HC: p<0.001). However similarly elevated levels were also present in ROU (0.95±1.01 ng/ml, p<0.05) and RA (1.1±23.3 ng/ml, p<0.01). Anti-HSP70 antibody levels were also significantly higher in BD (668±658 µg/ml) compared to HC (490±742.05 µg/ml, p<0.05). However no significant anti-HSP70 antibody responses were observed in ROU (634.7±548.21 µg/ml) and RA (431.8±840.98 µg/ml). No association of any organ manifestation, the disease duration, or treatment with HSP70 or anti-HSP70 antibody levels were observed in BD. A correlation between HSP70 and anti-HSP70 levels was only found in HC (p=0.007).*

Conclusions. *Free human HSP70 and anti-HSP70 antibodies are both elevated in patients with BD. HSP70-mediated innate and adaptive immune responses may participate in proinflammatory cytokine activation and tissue destruction in BD.*

Introduction

Heat shock proteins (HSPs) are a group of intra-cellular proteins that play a scavenger role for other intracellular proteins under denaturating stress conditions such as infections, hypoxia, trauma, and exposure to toxic drugs. Heat shock protein 70 (HSP70) is an intracellular molecular chaperone of naive, aberrantly folded, or mutated proteins and plays a cytoprotective role in stressful conditions (1). Significant sequence homology exists between the mammalian and microbial HSPs (mycobacterial and streptococcal HSPs have over 90% and human HSP 65/60s have over 50% homology) (2).

Among the various HSPs, 60/65 kDa HSP (HSP 65) in particular has been suggested as a candidate etiological agent for Behçet's disease (BD) (3-6). Recently, antibodies against other HSPs such as αβ-crystallin and HSP70 have also been demonstrated in the sera of BD patients (7, 8). This study was designed to investigate free HSP70 and anti-HSP70 levels in the serum of BD patients compared to rheumatoid arthritis (RA) and recurrent oral ulcer patients (ROU) and healthy controls (HC).

Materials and methods

Free human HSP70 levels were measured in the sera of 62 patients with BD [M/F: 23/39, mean age: 35.0±11.3 years; classified according to the ISG criteria (9); disease duration: 58.8±68.2 months] who were being followed in three outpatient clinics at Marmara University Medical School Hospital, Istanbul. All patients had oral ulcers. Genital ulcers were present in 79%, mucocutaneous lesions in 59%, arthritis in 33%, uveitis in 22%, vascular involvement in 20% and neurological disease in 4% of the patients. The pathergy test was positive in 70%. Sixty patients with RA (M/F: 7/53, mean age: 51.5±11.8 years, disease duration: 109.8±91.4 months), 37 patients with ROU (M/F: 16/21, mean age: 33.7±11.9 years, disease duration: 115.8±87.4 months), and 44 HC (M/F:

18/26, mean age: 31.3±9.3 years) were also examined.

Blood samples were collected from the patients and controls and the sera were stored at -20°C. Free HSP70 and anti-HSP70 antibody levels were determined using commercial ELİSA kits (Stressgen, Canada). Anti-HSP70 levels were measured in 40 patients with BD, 30 patients with ROU, 60 patients with RA, and in 44 healthy controls. The study was approved by the Faculty Research Ethical Committee and informed consent was obtained from all participants.

The data were analysed using the Kruskal-Wallis test and, for the subgroup analyses, Dunn's multiple comparisons test and the Mann-Whitney U-test. For the correlation analysis, Spearman's correlation test was applied.

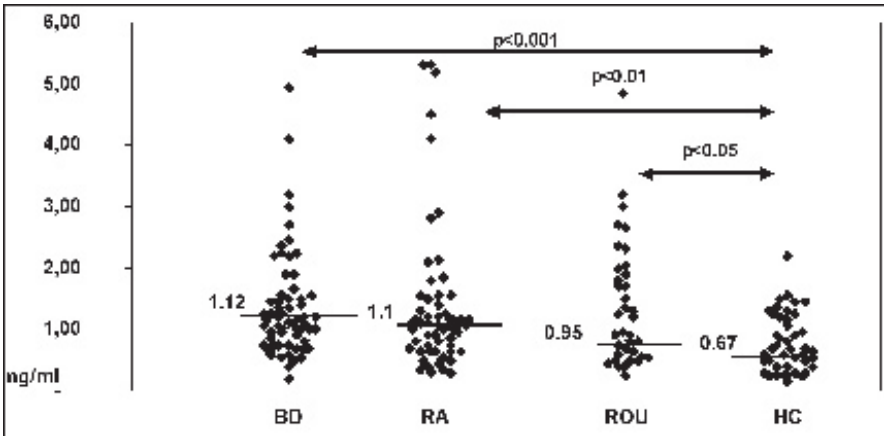

Fig. 1. Median free HSP70 levels in the sera.
BD *vs*. HC: $p<0.001$; ROU *vs*. HC: $p<0.05$; RA *vs*. HC: $p<0.01$; BD *vs*. RA, RA *vs*. ROU and BD *vs*. ROU: not significant. (BD: Behçet's disease; RA: rheumatoid arthritis; ROU: recurrent oral ulcer; HC: healthy controls).

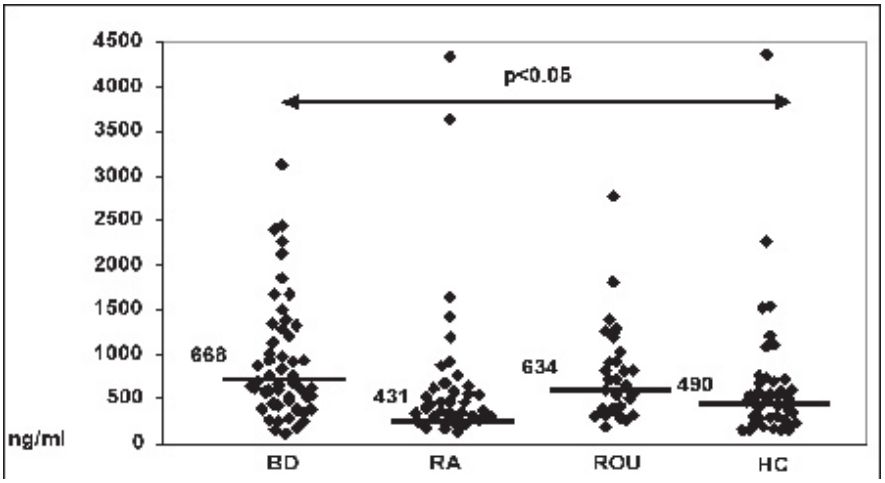

Fig. 2. Median anti-HSP70 levels in the sera.
BD *vs*. HC: $p<0.05$; BD *vs*. RA: $p<0.01$; BD *vs*. ROU, ROU *vs*. HC, RA *vs*. HC and RA *vs*. ROU: not significant. (BD: Behçet's disease; RA: rheumatoid arthritis; ROU: recurrent oral ulcer; HC: healthy controls).

Results

Median free HSP70 levels were significantly elevated in BD sera (1.12±0.86 ng/ml) compared to HC (0.67±0.46 ng/ml, BD *vs*. HC: $p<0.001$). However, similarly high levels were found in ROU (0.95±1.01 ng/ml, ROU *vs*. HC: $p<0.05$) and RA (1.1±23.28 ng/ml, RA *vs*. HC: $p<0.01$). There were no significant differences between BD and RA and ROU in the HSP70 levels (Fig. 1).

Median serum anti-HSP70 levels were also significantly elevated in BD (668±658 μg/ml) compared to HC (490±742.05 μg/ml: BD *vs*. HC: $p<0.05$) and RA (431.8±840.98 μg/ml, BD *vs*. RA: $p<0.01$). There were no significant differences in anti-HSP70 levels between BD and ROU (634.7±548.21 μg/ml), ROU and HC, ROU and RA, and RA and HC (Fig. 2).

When sub-group analyses were carried out based on family history, age, disease duration, disease activity, or therapy, no significant differences in either the HSP70 or the anti-HSP70 measurements were observed between the groups. A correlation between HSP70 and anti-HSP70 levels was observed only in HC ($p=0.007$).

Discussion

Heat shock proteins are highly conserved chaperone molecules with scavenger activity, which are involved in the correct folding of newly synthesized proteins (5). Chaperones are well-characterized proteins that participate in various stages of inflammation: initiating proinflammatory cytokine production, antigen recognition and processing; activating antigen-presenting cells; and chaperoning peptides during antigen presentation (10). HSP70 has been suggested to bind viral, bacterial, and tumor peptides, and acts like an adjuvant for immunogenicity (11). HSP70 can also be the initiator of a potent immune response (12). It was shown to bind to antigen-presenting cells (APC) and can generate various immune processes such as cytokine production (IL1β, TNF-α, IL-6, etc.), co-stimulatory molecule expression (CD86), and nitric oxide release. HSP70-induced proinflammatory cytokine production is mediated via the MyD88/IRAK/NF-κB signal transduction pathway and HSP70 utilizes both toll-like receptor-2 (TLR2) (receptor for gram-positive bacteria) and TLR4 (receptor for gram negative bacteria) to transduce its proinflammatory signal in a CD14-dependent fashion (13).

In this study we observed that HSP70 levels are elevated in BD, RA and ROU patients compared to healthy controls. There have been no previous studies investigating the levels of free HSP70 in the serum of BD patients. The source of free HSP70 in BD is currently

unknown, although necrotic or stressed cells have been shown to secrete HSP60 and HSP70 to their microenvironment. A similar cellular stress might also be the source of the HSP70 observed in our control patients with RA and ROU. Elevated free HSP70 levels in patients with BD might trigger anti-HSP70 immune responses; indeed, we found anti-HSP70 levels to be elevated, as would be expected in BD. Similarly, De Smet *et al*. reported elevated anti-HSP70 antibody levels in BD patients with uveitis (8). Although free HSP70 levels were also high in RA and ROU, anti-HSP70 levels were not significantly upregulated in these control groups compared to HC. However, low patient numbers might have led to a type II error for ROU in our study.

The augmented anti-HSP70 response may also be linked to innate responses in BD. We have recently showed that HSP60 upregulates TLR6 in BD neutrophils (14). The presence of HSP-activated neutrophils can affect the adaptive anti-HSP response more markedly in BD than in other inflammatory diseases.

In a recent study it was demonstrated that the presence of anti-HSP70 antibodies can enhance the production of CXCL-8 (IL-8) and tumor necrosis factor-alpha (TNFα) induced by HSP70 in human peripheral blood monocytes, possibly through TLR-4 (15). This enhancement is suggested to be due to the cross-linking of HSP70 by anti-HSP70 antibodies. Similarly, human sera with high anti-HSP60 antibody titers significantly enhanced the CXCL-8 production induced by human HSP60 in human monocytic cell lines. As CXCL-8 and TNFα both participate significantly in the inflammatory milieu in BD sera and tissue specimens, the presence of both HSP70 and anti-HSP70 antibodies in BD sera might have a prominent proinflammatory role in the pathogenesis of BD.

In conclusion, both free HSP70 and anti-HSP70 antibodies are upregulated in BD sera. Whether an HSP70-associated innate and adaptive immune activation participates uniquely in BD inflammation requires further study.

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