

# Serum levels of Heat Shock Protein-70 in Patients with Oral Lichen Planus

## SUMMARY

Usually increased presence of Heat Shock Proteins (HSPs) is considered to mediate inflammation process in Oral Lichen Planus (OLP) lesions, but in contrast HSP-70 expression found to be stable or even decreased in those lesions. The purpose of this study was to detect the serum titres of the HSP-70 in patients with OLP compared to healthy individuals, which may indicate an alternative, systemic role. Serum levels of HSP-70 were detected by sandwich-ELISA in 45 patients with reticular (n=28) and erosive (n=17) OLP, respectively. A group of 35 healthy individuals was used as control. HSP-70 was detected in significantly increased levels in OLP ( $p<0.05$ ) compared to controls. The increase was prominent in reticular-OLP ( $p<0.05$ ), whereas no difference was observed between serum HSP-70 in erosive OLP compared to controls. These results indicate a systemic initiation of the immune response in the pathogenesis and process of OLP. The higher titres of HSP-70 in reticular but not erosive form of OLP indicate rather an immunoregulatory role in chronicity than in the acute inflammatory process of OLP. Consequently, the evaluation of serum imbalances of HSP-70 in OLP using ELISA may be a useful marker for disease's monitoring and/or efficacy of systemic treatment.

**Keywords:** Heat Shock Protein 70 (HSP-70); Oral Lichen Planus

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## Introduction

Heat shock proteins (HSPs) are highly conserved proteins, essential for cell protection and survival, distributed in microorganisms and mammalian cells. They are induced by temperature as well as other physiological or pathological stressful events, acting as intracellular chaperones or as cytokine-like molecules<sup>1,2</sup>. Exogenous (from pathogens) and native (from host cells) HSPs can initiate innate and adaptive immunity, having a pro-inflammatory role, but they have also been considered as autoantigens *via* a cross-reactive immune response<sup>3,4</sup>.

HSP-70 is normally located within the cytoplasm as chaperone, playing a role in intracellular events (constitutively expression)<sup>2</sup>. Stress-inducible, extracellular or membrane HSP-70 participates in activation of the antigen-presentation process, cell recognition and enhancement of pro-inflammatory cytokines secretion by

monocytes, macrophages, and dendritic cells<sup>5</sup>. However, unlike other members of HSPs, HSP-70, also, seems to have an anti-proinflammatory effect, as shown in an experimental model of arthritis<sup>6</sup>.

Lichen planus is a chronic, inflammatory disease, involving mucosal surfaces and/or skin. Oral subtype of lichen planus (OLP) is the most characteristic immune-mediated oral disease related to cellular immunity against insufficient constituents of basal keratinocytes<sup>7</sup>. Several immunohistochemical studies showed increased presence of different HSPs members, in contrast to the non-altered or even decreased HSP-70<sup>8-12</sup> in OLP, as well as in cutaneous lichen planus<sup>13</sup>. Taking together the extended role of HSP-70 in immunity and its non-altered, even decreased, expression in involved tissues, this study investigates, for the first time, the serum levels of HSP-70 that may indicate an alternative systemic implication in OLP pathogenesis.

## Material and Methods

### Patients and Controls

45 OLP patients with clinical/microscopic confirmation, including 20 males and 25 females, aged from 27 to 75 years (mean age  $57 \pm 2.53$  years) participated in this study. 28 of the patients manifested the reticular and 17 the erosive form of OLP. All serum samples were obtained at the time of diagnosis and the patients had not received any treatment previously. Serum samples were also obtained from 35 age and sex matched (17 male and 18 female, mean age  $52.3 \pm 1.89$ ) healthy individuals (controls). All controls were free of infection and were not suffering from any autoimmune disease. The study was conducted according to Helsinki II declaration.

### HSP-70 ELISA

Serum Hsp-70 levels were assessed by colorimetric sandwich ELISA test according to manufacturers' instructions (Hsp70 ELISA, Stressgen Biotechnologies, Victoria, BC, Canada) as described in detail previously<sup>14</sup>. The test recognizes only inducible native and recombinant Hsp-70, but not other members of HSP family. The results were analysed using the SPSS v.18.0 statistical package. The unpaired Student's t-test was used for the correlation of HSP-70 titres of OLP patients and healthy controls. Significant levels of correlation were set at a *P* value of less than 0.05.

## Results

The serum HSP-70 values in the different forms of OLP and controls are presented in figure 1 and table 1. HSP-70 was detected in the serum of all patients and healthy individuals. Overall, titres of HSP-70 in OLP sera were significantly increased compared to controls ( $p=0.005$ ). Interestingly, when the comparison focused on the clinical form, only HSP-70 levels in reticular type of OLP cases were significantly increased compared to controls ( $p=0.0019$ ). In contrast, no differences of serum HSP-70 was observed between patients with erosive form of OLP and controls ( $p=0.17$ ).

Table 1. Mean  $\pm$  SE (ELISA) values\* of serum HSP-70 in OLP patients and controls

Patients with OLP	Controls	p
Total (n=45)	$3.8 \pm 0.4$	0.005
Reticular (n=28)	$2.04 \pm 0.3$	0.0019
Erosive (n=17)	$3.1 \pm 0.7$	0.17

\* Values =ng/mL

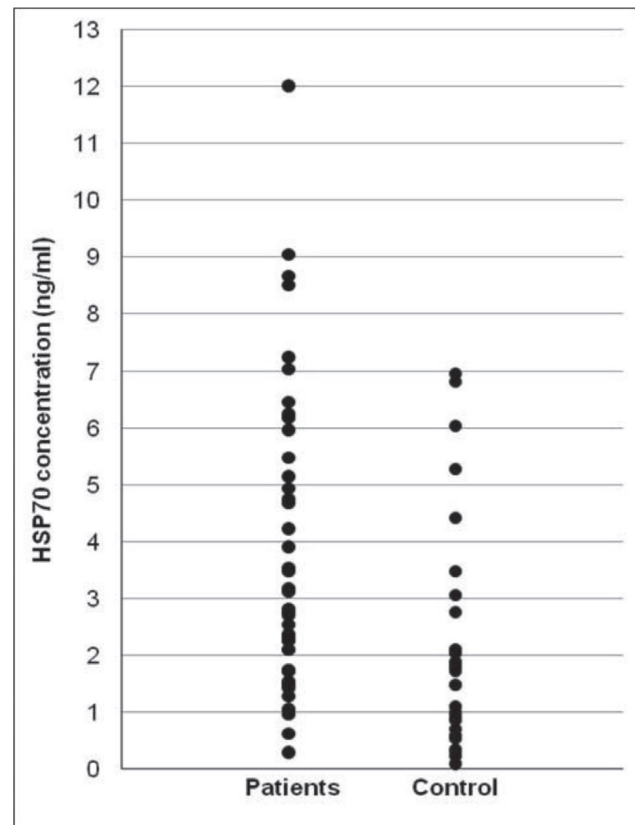


Figure 1. Serum (ELISA) HSP-70 values in OLP patients and controls

## Discussion

HSPs may have dual role, initiating immune-responses as inflammatory activators but also they can act as autoantigens *via* an immune cross-reaction response leading to the onset of immune-mediated diseases<sup>3,4</sup>. However, this self-HSPs reactivity has also been considered as a self-limited mechanism for regulation of pro-inflammatory process<sup>15</sup>.

Inducible HSP-70 may be presented as extracellular molecule or as cell membrane receptor or extracellular molecule<sup>16</sup>. It is believed to activate the innate immune response as antigen transporter, by triggering cytokines' secretion, and also as marker for both NK targeting and macrophage recognition/engulfment<sup>17</sup>. However, unlike other HSPs, HSP-70 seems to have an anti-inflammatory function also, as shown in an experimental model of arthritis<sup>6</sup>.

The link between HSP-70 and OLP could be supported by the ability of HSP-70 to mediate unknown antigen-presentation process and interaction between dendritic and T-cells *via* the expression of CD40L. Overall HSP-70 assists activation of both CD4+ T helper (for example by triggering the NF- $\kappa$ B-dependent activation of CD4+CD45RA memory T-cells) and cytotoxic CD8+ T cells, and consequently, increases cytokines' expression. Also, this HSP mediates the

apoptosis induction<sup>18,19</sup>. These phenomena are hypothesized to be involved in the pathogenesis of OLP<sup>7</sup>.

However, in contrast to Sugerma et al (1995)<sup>8</sup>, who found significant increase of HSP-70, other studies in involved oral mucosal<sup>10,11</sup> and cutaneous lichen planus lesions<sup>13</sup> showed downregulation or non significant difference<sup>9,12</sup>. Indeed, based on the immunohistochemical data, it is questionable, whether a local apoptotic, pro-inflammatory role of HSP70 in lichen planus is truly evident. It can be hypothesized that, due to stress-induced gene dysfunction, HSP-70 of oral keratinocytes may act as autoantigen or assist other (unknown) antigen-recognition, before other shock proteins mediate the “outbreak” of inflammation as part of cellular immune response, in a very early phase of pathogenesis. Unfortunately, in this early step, the exact expression pattern of HSP-70 cannot be, easily, detected, compared to its normal even downregulated expression in inflammatory phase.

The increased levels of serum HSP-70 in OLP, as shown for the first time in our study, possibly represent a “fingerprint” of a generalized immune response occurring in immune-mediated diseases, similarly to recurrent aphthous ulceration or Behcet disease<sup>20</sup>. The significant increase of serum HSP-70 in reticular form of OLP indicates a role in the systemic regulation of immune response, actually in self-limitation, and “chronicity” of OLP, but not in the expansion of tissue inflammation. Further investigation regarding the possible serum and/or saliva imbalance of HSP-70 in OLP, evaluated by ELISA, could be useful marker for monitoring of the disease, systemic treatment efficacy and early prediction of recurrences.

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