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Circulating Heat Shock Proteins in Women With a History of Recurrent Vulvovaginitis

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Abstract

Objective: Predisposing factors influencing recurrences of bacterial vaginosis (BV) or vaginitis from *Candida* remain unidentified for most women. As a component of studies to determine host susceptibility factors to genital tract infections in women, we measured expression of the 60-kDa and 70-kDa heat shock proteins (hsp60 and hsp70, respectively) in the circulation of women with or without a history of recurrent BV or candidal vaginitis and with or without a current lower genital tract infection. Heat shock protein expression is associated with a down-regulation of pro-inflammatory immune responses that would inhibit microbial infection.

Method: The investigators measured hsp60 and hsp70, antibodies to these proteins, the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), and the anti-inflammatory cytokine interleukin-10 (IL-10) in sera by ELISA. The study population consisted of 100 women who attended a gynecology clinic in Campinas, Brazil. Of those, 55 had a history of recurrent vulvovaginitis (RV), while 45 were controls with no such history. Only women who were asymptomatic for at least 1 month were studied.

Results: Although all were asymptomatic, clinical and microbiological examination revealed that five of the women with a history of RV and two controls had a current candidal vaginal infection; 16 RV patients and 12 controls had BV; and six RV patients had both BV and candidiasis. Twenty-eight RV patients and 31 controls had no clinical or microbiological detectable vaginal infection. Among the RV patients, hsp60 and hsp70 were more prevalent in those with current BV (40.9% and 50.0%, respectively) or a candidal infection (45.5% and 54.5%) than in women with no current infection (21.4% and 17.9%). In the women with no history of RV, BV was not associated with a high prevalence of hsp60 (8.3%) or hsp70 (8.3%). Interleukin-10 and TNF were not more prevalent in vaginitis patients or controls with a current candidal infection or BV than in uninfected subjects.

Conclusion: The high prevalence of circulating hsp60 and hsp70 in women with a history of RV and current BV or vaginal candidiasis, but not in women with no history of RV, suggests that differences in heat shock protein induction may be related to susceptibility to recurrent vaginal infections. Infect. Dis. Obstet. Gynecol. 7:128–132, 1999. © 1999 Wiley-Liss, Inc.

Key words
heat shock proteins; vaginitis; bacterial vaginosis; candidiasis

Frequent recurrences of vaginitis caused by *Candida* or bacterial vaginosis (BV) occur only in a subgroup of women following an initial episode of either infection. Why most women remain free of symptoms for long periods after appropriate antibiotic treatment while the infections return shortly

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Clinical Study

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after cessation of therapy in others remains unexplained.

We have been examining host factors as determinants of susceptibility to infections and their consequence in the female genital tract. As a component of these investigations the presence of the 60-kDa and 70-kDa heat shock proteins (hsp60 and hsp70, respectively) in sera was determined. Hsp60 and hsp70 are highly conserved proteins present in organisms ranging from bacteria to humans. Under normal conditions, they function in the assembly, folding, and transport of polypeptides. Synthesis of both proteins is greatly accelerated under nonphysiological conditions to prevent incorrect protein aggregation or denaturation.[1] Enhanced heat shock protein expression is, thus, a sensitive indicator of nonphysiological conditions.

Transcription of heat shock protein genes is also coupled to the down-regulation of pro-inflammatory cytokine expression. Both interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) transcription is inhibited by the stimulation of heat shock protein gene activation.[2–4] Since IL-1 and TNF-α are involved in initiation of immune responses, inhibition of their synthesis would impair an organism's immune defense. Therefore, expression of heat shock proteins might be associated with diminished immunity and increased susceptibility to infection.

In this communication we report on the high prevalence of hsp60 and hsp70 in the circulation of women with a history of recurrent vaginal infections (RV) and a current case of either candidal infection or BV, but not in those currently free of disease or in women with a current infection but no history of recurrent vaginitis.

MATERIALS AND METHODS

Subjects

The study population consisted of 100 nonpregnant, women of reproductive age who attended a gynecology clinic in Campinas, Brazil. Fifty-five of the women had at least three episodes of either candidal vaginitis or BV in the past year, as determined from medical records. The diagnostic criteria for these infections are stated below. The other 45 women had one or no vaginal infections in the previous 12 months. The vaginal infections and control groups were similar in age (30.09 ± 7.9 years in the infected group vs. 32.21 ± 7.7 years in the control group), socioeconomic status, and racial composition. In an effort to differentiate predisposing factors from those associated with current clinical infection, only subjects who were clinically asymptomatic (no reports of itching, burning, discharge, or soreness during intercourse) at the time of sample collection and for at least 1 month prior were studied.

The study was approved by the Scientific and Ethics Committee for Research at the University of Campinas, Brazil.

Microbiological Diagnosis

The vaginal microbiology of each subject was assessed by wet mount and Gram stain. Patients were not menstruating and had not had intercourse for at least 24 hours prior to the visit. Bacterial vaginosis was diagnosed clinically by the presence of three of the following: vaginal pH > 4.5, presence of a homogeneous grey/white discharge, detection of clue cells, amine odor upon addition of potassium hydroxide to secretions. The presence of *Candida* was assessed by wet mount, Gram stain, and culture in Sabouraud medium.

Serum Samples

Blood was obtained by venipuncture, allowed to clot, and sera was collected by centrifugation and stored at −20°C until shipped to New York on dry ice for analysis.

Heat Shock Protein Detection

Hsp60 and hsp70 were detected in sera by ELISA as described[5–7] utilizing specific monoclonal antibodies bound to wells of a microtiter plate as the capture antibody and polyclonal antibody as detecting antibody (StressGen, Victoria, British Columbia). The values were converted to μg/mL by reference to a standard curve generated with purified recombinant hsp60 and hsp70 (StressGen). The lower limit of sensitivity was 10 ng/mL for both proteins.

Antibodies to Heat Shock Proteins

Circulating immunoglobulin G (IgG) antibodies to hsp60 and hsp70 were detected by ELISA as previously described[8,9] using purified recombinant hsp60 and hsp70 (StressGen) bound to wells of a microtiter plate. Known positive and negative sera were always assayed in parallel to the test samples.

TABLE 1. Vaginal infections in study subjects

Infection	Number of subjects (%)	
	History of recurrent vaginal infections	No history of recurrent vaginal infections
Candida[a]	5 (9.1)	2 (4.4)
Bacterial vaginosis (BV)[b]	16 (29.1)	12 (26.7)
Candida plus BV	6 (10.9)	0
None	28 (50.9)	31 (68.9)

[a]*Candida* was detected by wet mount, Gram stain, and culture.
[b]BV was detected by clinical criteria.

A sample was defined as positive for IgG antibodies if it yielded an absorbance more than two standard deviations above the mean value obtained with a panel of negative control sera.

Cytokine Detection

The presence of interleukin-10 (IL-10) and TNF-α in sera was measured by commercial ELISA (BioSource, Canarillo, CA). The value were converted to pg/mL by reference to a standard curve generated with purified cytokines. The lower limit of sensitivity was 10 pg/mL.

Statistics

Fisher exact test was used to analyze differences between groups in heat shock protein and cytokine expression. Spearman rank correlation test was used to analyze the relation between different assays.

RESULTS

Vaginal Infections

Although all subjects were asymptomatic for at least the previous 30 days, they were not all free from vaginal infections (Table 1). Bacterial vaginosis was identified in 16 (29.1%) of the RV patients and 12 (26.7%) controls; five (9.1%) RV patients and two (4.4%) controls had vaginal candidiasis, while an additional six (10.9%) RV patients and no controls had both BV and candidiasis. No infections were evident in 28 (50.9%) RV patients and in 31 (68.9%) controls. None of the differences between RV patients and controls were statistically significant.

Heat Shock Protein Detection

Among the 55 women with a history of RV, 19 (34.5%) were seropositive for hsp70 (Table 1)

TABLE 2. Hsp60 and hsp70 in sera of women with a history of recurrent vaginal infections[a]

Current infection	No. subjects	No. positive (%)	
		hsp60	hsp70
None	28	6 (21.4)	5 (17.9)
Candida[b]	11	5 (45.5)	6 (54.5)[c]
BV[b]	22	9 (40.9)	11 (50.0)[d]

[a]BV, bacterial vaginosis.
[b]Six women were positive for both *Candida* and BV.
[c]$P = 0.04$ vs. no infection.
[d]$P = 0.03$ vs. no infection.

Those with current BV or candidiasis had a higher percentage of positive sera (50.0% and 54.5%, respectively) than did women with no current infection (17.9%). The individual values are shown in Fig. 1.

Similarly, 17 (30.9%) of the RV patients were seropositive for hsp60 antigen. However, the RV subjects with current BV or current candidiasis had a higher percentage of hsp60-positive sera (40.9% and 45.5%, respectively) than did women with a past history of RV but with no current infection (21.4%) (Table 2, Fig. 1).

In marked contrast, among the women with no history of RV but with current BV, only one (8.3%) was positive for hsp60 ($P < 0.05$ vs. RV group with current BV) and only one (8.3%) was positive for hsp70 ($P < 0.05$). Only two women in the control group had current candidiasis; neither was hsp60-positive, and one was hsp70-positive. There was no difference in heat shock protein expression between RV patients and controls with no current infection.

There was no relation between hsp60 or hsp70 and method of contraception or history of sexually transmitted diseases. In both the control and RV groups the presence of hsp60 and hsp70 were highly correlated ($P < 0.0001$).

Cytokine Analysis

Among the women with a history of RV, IL-10 was present in one (9.1%) with current candidiasis, eight (36.4%) women with BV, and five (17.9%) of those with no current vaginal infection. Among the control women, four (33.3%) with current BV were IL-10-positive, as were 12 women (38.7%) with no current infection. None of these differences were statistically significant.

Similarly, TNF-α was unrelated to current in-

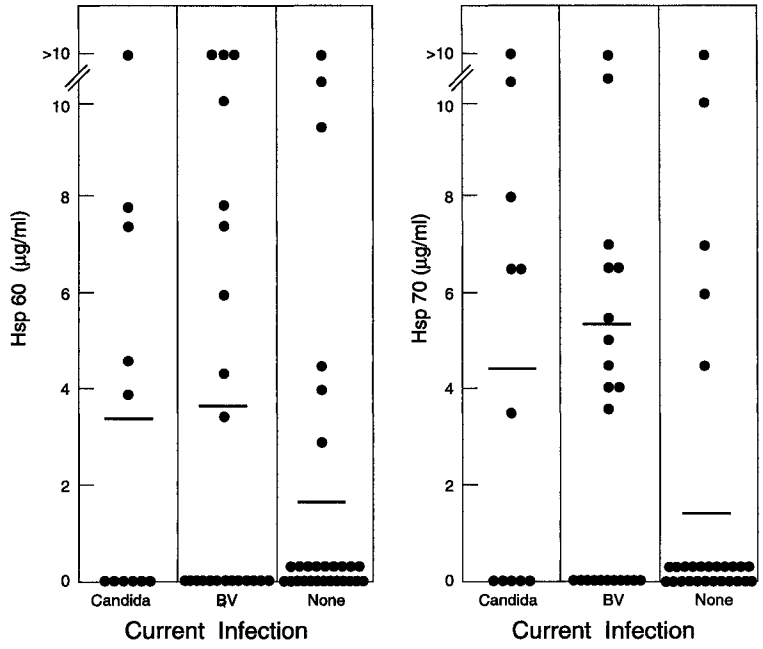

Fig. 1. Circulating heat shock proteins in women with a history of recurrent vaginal infections, with or without a current vaginal infection. Sera from 55 women with current candidal infection or BV were tested for hsp60 and hsp70 by ELISA. Values were converted to µg/mL by reference to a standard curve. Six women were positive for both *Candida* and BV. The horizontal line indicates the mean value.

fection in women with past RV. Tumor necrosis factor-α was detected in 18.2% of those with current candidiasis, 22.7% of those with current BV, and 21.4% of women with no current infection. Among the controls, 25% of women with BV and 6.5% with no current infection were positive for TNF-α. All cytokine results are presented in Table 3.

Antibodies to Heat Shock Proteins

The IgG antibodies to hsp60 were identified in nine (16.6%) women with past RV and in six (13.3%) controls. Similarly, IgG anti-hsp70 was present in eight (14.5%) RV patients and in three (6.7%) controls. In contrast to the heat shock protein antigen results, there were no differences between patients and controls, or between the presence or absence of a current infection, and heat shock protein immunity.

DISCUSSION

In a previous smaller study, we demonstrated that hsp60 and hsp70 were present in the vaginas of asymptomatic women with a history of recurrent candidal vaginitis or recurrent BV, but not in control women.[7] The present investigation on a larger population confirms the association between heat shock protein expression and history of RV and, furthermore, reveals that the appearance of both hsp60 and hsp70 in the circulation of those patients corresponds to either a current candidal infection or BV.

TABLE 3. Cytokine production in relation to current infection in women with or without a history of recurrent candidal vaginitis or BV

	Control		Recurrent infection	
Subjects	IL-10	TNF-α	IL-10	TNF-α
All				
No. positive	17/45	5/45	13/55	11/55
Cytokines (pg/mL)	15–38	16–68	12–48	37–831
Current BV				
No. positive	4/12	3/12	8/22	5/22
Cytokines (pg/mL)	28–38	17–68	12–46	33–105
Current *Candida*				
No. positive	1/2	0/2	1/11	2/11
Cytokines (pg/mL)	37	0	12	65–105
No current infection				
No. positive	12/31	2/31	5/28	6/28
Cytokines (pg/mL)	15–30	16–66	21–48	64–81

Heat shock protein expression was not associated with the presence of antibodies to hsp60 or hsp70. This is consistent with previous studies on the low incidence of circulating antibody to heat

shock proteins in healthy women[8] and suggests that heat shock protein-related immunity is not a risk factor for recurrences of vaginitis or BV.

The Hsp60 antigen previously has been demonstrated in genital tract secretions from men with sperm autoantibodies in association with *Chlamydia trachomatis* infection.[5] Similarly, hsp60 has been identified in follicular fluids from infertile women with chlamydial infection.[10] The stress response associated with a genital tract infection appears to be sufficient to induce readily detectable levels of heat shock proteins.

Heat shock protein expression in the vagina was followed by the appearance of hsp60 and hsp70 in the circulation. There is ample precedent for the migration of substances from the vagina to the systemic circulation.[11–13] The data are consistent with the hypothesis that there are differences between women with a history of RV and other women in the inducibility or extent of induction of heat shock protein expression following genital tract infection. Studies to examine whether polymorphisms in heat shock protein and immune regulatory genes in these women are responsible for differences in hsp60 and hsp70 gene expression are now in progress.

The relation between heat shock protein expression and the mechanism of recurrent candidal vaginitis or BV remains to be determined. One possibility is that induction of heat shock protein gene transcription is more readily induced in the vagina in these patients than in other women and that the concomitant inhibition of transcription of genes associated with induction of cellular immunity results in a decreased ability to inhibit the growth of pathogenic microorganisms in the vagina. The failure in the present study to detect differences in serum TNF-α and IL-10 levels among groups suggests that these samples might not be sufficiently concentrated for accurate analysis. In an earlier study, vaginal hsp60 and hsp70 was associated with IL-10, an immunosuppressive cytokine.[7]

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