Abstract. Background: Heat-shock protein 90 (HSP90) is an abundant protein in mammalian cells. It interacts with a variety of proteins that play key roles in breast neoplasia. This is the first study to assess serum levels of HSP90 in atypical ductal hyperplasia (ADH), lobular neoplasia (LN), ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC) and infiltrative lobular carcinoma (ILC). Patients and Methods: Serum concentrations of HSP90 in women with benign (n=34), ADH (n=26), DCIS (n=30), IDC (n=29), LN (n=20) and ILC (n=9) lesions were determined with immunoenzymatic assays. For the evaluation of serum concentrations along the transition from benign through precursor and preinvasive to invasive lesion, the severity of diagnosis was treated as an ordinal variable. Results: No significant association was demonstrated between serum HSP90 levels and the severity of the lesion in ductal and lobular series. The post hoc comparison between the lobular and ductal precursor lesions (i.e. ADH vs. LN) did not yield a statistically significant difference. Similarly, the post hoc comparison between the lobular and ductal invasive carcinomas (i.e. IDC vs. ILC) did not point to a statistically significant difference. Conclusion: This is the first study evaluating HSP90 serum levels in both lobular and ductal lesions of the breast. Contrary to published pathological findings according to which HSP90 exhibits significant variability along both series, such a finding was not replicated for the level of serum HSP90 concentrations.

Heat-shock protein 90 (HSP90) is an abundant protein in mammalian cells (1). It forms several discrete complexes, each containing distinct groups of co-chaperones that assist protein folding and refolding during stress, protein transport and degradation (2). HSP90 interacts with a variety of proteins that play key roles in breast neoplasia, including estrogen receptors (ER), tumor suppressor p53 protein, angiogenesis transcription factor, hypoxia-inducible factor 1α (HIF-1α), antiapoptotic kinase AKT, RAF-1, MAP kinase and a variety of receptor tyrosine kinases of the ERBB family (reviewed in (3)). Elevated HSP90 expression has been documented in breast ductal carcinoma tissue (4-8) to contribute to the proliferative activity of breast cancer cells; significantly reduced HSP90 expression has been shown in infiltrative lobular carcinoma and lobular neoplasia (9, 10). HSP90 overexpression has been proposed to be a component of a mechanism through which breast cancer cells become resistant to various stress stimuli (6). Given the above observation, it would appear that pharmacological inhibition of HSPs can provide therapeutic opportunities in the field of cancer treatment (11-15); 17-allylamino,17-demethoxygeldanamycin (17-AAG) is the first HSP90 inhibitor to be clinically investigated and has yielded promising results (3, 16).

Precursors and preinvasive lesions of the breast, which include atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), and lobular neoplasia (LN), represent several heterogeneous entities (17-19). Over the past twenty years, a histological model of human breast cancer evolution has prevailed (20, 21) supporting there being a transition from normal epithelium to invasive carcinoma via non-atypical hyperplasia, ADH and DCIS (22, 23). LN is a ‘marker of increased risk’ rather than a true precursor of invasive carcinoma. The term LN refers to the entire spectrum of atypical epithelial proliferations originating in the terminal duct-lobular unit, with or without pagetoid involvement of terminal ducts (24, 25).
Table I. Serum concentrations of HSP90 in benign disease, (Atypical Ductal Hyperplasia), (Ductal Carcinoma in situ) and (Invasive Ductal Carcinoma). Values are expressed as the mean (standard deviation).

|                | Benign (n=34) | ADH (n=26) | DCIS (n=30) | IDC (n=29) | p-Value
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<td>Hsp90 (ng/ml)</td>
<td>17.3 (11.8)</td>
<td>18.8 (13.5)</td>
<td>18.8 (17.7)</td>
<td>18.1 (14.1)</td>
<td>0.590</td>
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†p-Values derived from Spearman’s rank correlation coefficient.

There is a marked scarcity of data with respect to the serum expression of HSP90 in precursor, preinvasive and invasive breast lesions and its prognostic role. This is the first study to assess serum levels of HSP90 in ADH, LN, DCIS and invasive ductal carcinoma (IDC); special attention has been paid to make the distinction between ductal (ADH, DCIS, IDC) and lobular (LN; invasive lobular carcinoma ILC) series clear.

Patients and Methods

Study population. The study included 148 women; 26 with ADH, 30 with DCIS, 29 with IDC, 20 with LN, 9 with ILC, and 34 with benign breast lesions. The mean age of the patients was 53.7±9.8 years. The patients underwent vacuum-assisted breast biopsy, excisional breast biopsy, lumpectomy or modified radical mastectomy, subsequently followed by histopathological diagnosis of the lesion. Patients bearing mixed lesions with co-existent disease were excluded from the study. Benign lesions were randomly selected from our total patient pool in parallel.

Prior to their enrollment, all the participants were evaluated for the absence of diabetes mellitus, stroke, autoimmune diseases, history of other cancer, coronary heart disease, psychiatric conditions, asthma and infection currently or within the last two months. The participants were recruited from the Breast Unit, First Department of Propaedeutic Surgery, Athens University Medical School at Hippokратio Hospital between September 2008 and June 2010. The Institutional Research Committee approved the protocol and consent forms were obtained from all participants prior to study entry. The histopathological data were reported during the statistical analysis, after the blind completion of the assays.

Sample analysis. Peripheral venous blood samples were collected between 12:00 a.m. and 14:00 p.m. prior to biopsy or surgery. Serum and plasma samples were isolated from whole blood by centrifugation according to standard protocols, and stored at -80°C in aliquots. Serum HSP90a levels were determined in duplicate by a solid-phase ELISA technique (Assay Designs, Stressgen, MI, USA).

Statistical analysis. For the evaluation of serum concentrations along the benign to invasive transition, the severity of diagnosis was treated as an ordinal variable. More specifically, the variable corresponding to the severity of the lesion was expressed on a 1-4 scale regarding ductal lesions [1: benign lesion, 2: precursor lesion (ADH), 3: DCIS, 4: IDC] and on a 1-3 scale regarding lobular lesions [1: benign lesion, 2: precursor lesion (LN), 3: ILC]. For serum HSP90 levels, the associations with lesion severity were evaluated by means of the Spearman’s rank correlation coefficient. Similarly, the association between serum HSP90 levels and patients’ age was evaluated by the same test.

Additionally, two post hoc analyses were conducted, one assessing differences in HSP90 between the precursor lesion subpopulations (ADH vs. LN) and another evaluating differences between the invasive carcinoma subpopulations (IDC vs. ILC). Mann-Whitney-Wilcoxon test for independent samples (referred to as ‘MWW’ for reasons of brevity) was performed in these subanalyses.

STAIA 11.1 statistical software was used for the statistical analysis (StataCorp, College Station, TX, USA).

Results

Table I presents serum concentrations of HSP90 along the continuum of ductal lesions; no significant association was demonstrated between serum HSP90 levels and the severity of the lesion (Spearman’s rho=–0.050, p=0.590). Table II presents the respective data along the lobular series; similarly, the association between serum HSP90 levels and the severity of the lesion was not statistically significant (Spearman’s rho=–0.018, p=0.890).

The mean (±standard deviation) patient age was 53.7±9.8, (range: 35-80) years. Serum HSP90 levels were not correlated to patient age (Spearman’s rho=0.056, p=0.503).

Worthy of note, the post hoc comparison between the lobular and ductal precursor lesions (i.e. ADH vs. LN) did not yield a statistically significant result (p=0.965, MWW). Similarly, the post hoc comparison between the lobular and ductal invasive carcinomas (i.e. IDC vs. ILC) did not point to there being a statistically significant difference (p=0.503, MWW).

Discussion

This is the first study evaluating HSP90 serum levels in both lobular and ductal lesions of the breast. Contrary to the pathological findings published by our team (8-10), as well as by other research groups (5-7, 11), according to which tissue expression of HSP90 exhibits significant variability along both series, such a finding was not replicated for serum concentrations of HSP90.
HSP90 (ng/ml) 17.3 (11.8) 19.2 (16.2) 19.7 (12.0) 0.890

$p$-Values derived from Spearman’s rank correlation coefficient.

Previous pathological research has demonstrated increasing tissue levels of HSP90 along the progression of ductal lesions (8), whereas the opposite pattern has been uncovered in pathological specimens derived from lobular series (9, 10). As a result, it has been postulated that series-specific features may interfere with the immunohistochemically evaluated expression of HSP90 (8-10). Nevertheless, once again, our post hoc subanalyses did not point to there being any differences between lobular and ductal lesions, either at the level of precursor lesions or at the level of invasive carcinomas.

The potential explanation underlying this discrepancy from the published literature essentially presupposes some quantitative aspects of HSP90 biology. HSP90 is a ubiquitous protein, comprising as much as 1-2% of total cellular protein content; interestingly enough, this amount may well increase by about twofold under stress conditions (26). HSP90 mediates essential housekeeping functions, such as de novo protein folding, translocation of proteins across membranes, quality control in the endoplasmic reticulum and normal protein turnover (26). As a result, it seems that any protein overexpression by a single breast lesion may not be capable of modifying the total, constitutively and ubiquitously produced, large amounts of endogenous HSP90.

Our null finding may be of particular importance, since recent phase II trials assessing HSP90 inhibitors are expected to yield promising results. Thus, measurement of serum HSP90 as a reliable surrogate endpoint may be of special value. Our results do not seem to be in accordance with other studies suggesting a role of serum HSP90 as a marker of neoplasia, for example in hepatocellular cancer (27). It appears that instead of HSP90, HSP70 might be a more useful biomarker for trials on HSP90 inhibitors, as postulated by a recent study (28). Other biomarkers, such as insulin-like growth factor binding protein-2 and HER-2 extracellular domain, have exhibited satisfactory properties in experimental animal models for assessing resistance to HSP90 inhibitors (29).

Despite the originality of this study, some limitations should be mentioned. It should be declared that the retrieval of ‘pure’ ADH and LN lesions i.e., without coexistence of carcinomas is difficult, as these lesions represent rare entities. This fact can rationalize the sample size of the present study. On the other hand, the strict selection criteria that were adopted here may be considered assets of this study, as conditions possibly modifying HSP90 levels have been excluded, so as to yield a clear picture of HSP90 patterns in breast carcinogenesis.

In conclusion, serum HSP90 concentration does not seem to be modified along the progression of either ductal or lobular lesions of the breast. Longitudinal studies investigating the profile of serum HSP90 concentration after surgery, chemotherapy and adjuvant treatment seem valuable for the extrapolation and confirmation of the presented results.

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References


