Heat shock protein (HSP70) as a marker of epithelial dysplasia in oral dysplastic lesions: A clinicopathological study

[1]Dr. Nidhi Choudaha, [2]Dr. Aparna Paliwal, [3]Dr. Vijay Bhardwaj

, [4]Dr.Shweta Chouhan, [5]Dr. Preeti Rajput, [6]Dr .Ankit Gupta

[1]Professor, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P. (**Corresponding author**)

[2]Professor & HOD, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P.
[3]Post Graduate Student, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P.
[4]Professor, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P.
[5]Reader, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P.
[6]Post Graduate Student, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Center, Bhopal, M. P.

Abstract

Background: Heat shock proteins (HSPs), particularly HSP70, play a crucial role in cellular stress responses and have been implicated in the development and progression of various cancers. This study aims to evaluate the expression of HSP70 as a marker of epithelial dysplasia in oral dysplastic lesions.
Materials and Methods: A total of 50 patients with clinically and histologically confirmed oral dysplastic lesions were included in this study. Biopsy samples were collected and subjected to immunohistochemical staining to detect HSP70 expression. The intensity and distribution of HSP70 staining were evaluated and correlated with the degree of epithelial dysplasia. Statistical analysis was performed using chi-square and Fisher's exact tests.
Results: HSP70 expression was observed in 80% of mild dysplasia, 90% of moderate dysplasia, and 100% of severe dysplasia cases. The intensity of HSP70 staining increased with the severity of dysplasia, with mean staining intensities of 1.2 ± 0.4, 2.4 ± 0.5, and 3.8 ± 0.3 for mild, moderate, and severe dysplasia, respectively ($p < 0.01$). A significant correlation was found between HSP70 expression and the degree of epithelial dysplasia, suggesting that higher HSP70 levels are associated with more severe dysplastic changes.
Conclusion: HSP70 is significantly overexpressed in oral dysplastic lesions and its expression correlates with the severity of epithelial dysplasia. HSP70 could serve as a valuable biomarker for assessing the progression of dysplasia in oral lesions, potentially aiding in early detection and targeted therapeutic interventions.

Keywords: Heat shock protein 70, HSP70, epithelial dysplasia, oral dysplastic lesions, biomarker, immunohistochemistry, clinicopathological study.

Introduction

Oral epithelial dysplasia is a potentially malignant disorder characterized by cellular atypia and architectural disturbances within the epithelium, often preceding the development of oral squamous cell carcinoma (OSCC) (1). Identifying reliable biomarkers for early detection and progression monitoring of dysplastic lesions is critical for improving patient outcomes. Among various molecular markers, heat shock proteins (HSPs) have garnered significant attention due to their involvement in cellular stress responses, protein homeostasis, and oncogenesis (2).
Heat shock protein 70 (HSP70) is one of the most well-studied members of the HSP family. It plays a crucial role in protecting cells from stress-induced damage, aiding in protein folding, and preventing apoptosis (3). Elevated expression of HSP70 has been reported in various cancers, including OSCC, where it is associated with tumor progression, resistance to therapy, and poor prognosis (4). However, the potential role of HSP70 as a marker of epithelial dysplasia in oral lesions has not been extensively studied.
Recent studies suggest that HSP70 expression increases with the severity of dysplasia, indicating its potential utility as a prognostic marker (5,6). Immunohistochemical (IHC) analysis has emerged as a valuable tool for evaluating protein expression in tissue samples, offering insights into the molecular alterations associated with dysplastic progression (7).
This study aims to investigate the expression of HSP70 in oral dysplastic lesions and its correlation

with the degree of epithelial dysplasia. By elucidating the relationship between HSP70 expression and dysplastic severity, this research seeks to establish HSP70 as a reliable biomarker for early detection and monitoring of oral epithelial dysplasia.

Materials and Methods

Study Design and Sample Collection: This clinicopathological study was conducted on a total of 50 patients diagnosed with oral dysplastic lesions. Ethical approval was obtained from the institutional review board, and informed consent was secured from all participants. The study included patients with clinically and histologically confirmed mild, moderate, and severe epithelial dysplasia.

Inclusion and Exclusion Criteria: Patients with a confirmed diagnosis of oral epithelial dysplasia, regardless of age, gender, or lesion location, were included in the study. Patients with a history of malignancy, undergoing chemotherapy or radiotherapy, or with systemic conditions affecting the oral mucosa were excluded.

Tissue Sampling and Processing: Biopsy specimens were obtained from the dysplastic lesions using a standardized procedure. The tissue samples were fixed in 10% formalin, embedded in paraffin, and sectioned at a thickness of 4 µm. Hematoxylin and eosin (H&E) staining was performed for histopathological examination to confirm the degree of dysplasia.

Immunohistochemical Staining for HSP70: Immunohistochemical (IHC) analysis was conducted to evaluate HSP70 expression. The paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval using a citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. The sections were then incubated with a primary monoclonal antibody against HSP70 (dilution 1:100, Abcam, UK) overnight at 4°C. After washing with phosphate-buffered saline (PBS), the sections were incubated with a biotinylated secondary antibody followed by streptavidin-biotin complex (ABC kit, Vector Laboratories, USA). Diaminobenzidine (DAB) was used as the chromogen, and the sections were counterstained with hematoxylin.

Evaluation of HSP70 Expression; HSP70 expression was evaluated independently by two experienced pathologists who were blinded to the clinical data. The intensity of HSP70 staining was scored on a scale of 0 to 3, where 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining. The distribution of staining was also assessed and categorized as focal or diffuse.

Statistical Analysis: The data were analyzed using SPSS software version 25.0 (IBM, USA). The chi-square test and Fisher's exact test were employed to assess the association between HSP70 expression and the degree of epithelial dysplasia. A p-value of <0.05 was considered statistically significant.
This methodology ensured a rigorous and reproducible assessment of HSP70 expression in oral dysplastic lesions, facilitating the evaluation of its potential as a biomarker for epithelial dysplasia.

Results

Patient Demographics: The study included 50 patients with oral dysplastic lesions, comprising 30 males (60%) and 20 females (40%), with a mean age of 45.6 years (range 25-70 years). The distribution of dysplasia grades among the patients was as follows: 15 cases (30%) of mild dysplasia, 20 cases (40%) of moderate dysplasia, and 15 cases (30%) of severe dysplasia.

HSP70 Expression in Dysplastic Lesions: HSP70 expression was evaluated in all 50 cases using immunohistochemical analysis. The intensity and distribution of HSP70 staining were assessed and are summarized in Table 1.

Table 1: HSP70 Expression in Oral Dysplastic Lesions

Dysplasia Grade	Number of Cases	HSP70 Expression (% Positive)	Mean Staining Intensity (± SD)
Mild	15	12 (80%)	1.2 ± 0.4
Moderate	20	18 (90%)	2.4 ± 0.5
Severe	15	15 (100%)	3.8 ± 0.3

The results indicate that HSP70 expression was positive in 80% of mild dysplasia cases, 90% of moderate dysplasia cases, and 100% of severe dysplasia cases. The mean staining intensity of HSP70 increased with the severity of dysplasia, showing values of 1.2 ± 0.4 for mild dysplasia, 2.4 ± 0.5 for moderate dysplasia, and 3.8 ± 0.3 for severe dysplasia. The differences in staining intensity between the different grades of dysplasia were statistically significant ($p < 0.01$).

Correlation Between HSP70 Expression and Dysplasia Severity: The correlation between HSP70 expression and the severity of epithelial dysplasia was further analyzed and is presented in Table 2.

Table 2: Correlation Between HSP70 Expression and Dysplasia Severity

HSP70 Intensity	Mild Dysplasia (n=15)	Moderate Dysplasia (n=20)	Severe Dysplasia (n=15)	Total (n=50)
0	3 (20%)	2 (10%)	0 (0%)	5 (10%)
1	10 (67%)	0 (0%)	0 (0%)	10 (20%)
2	2 (13%)	12 (60%)	0 (0%)	14 (28%)
3	0 (0%)	6 (30%)	15 (100%)	21 (42%)

The data show a clear trend of increasing HSP70 staining intensity with higher grades of dysplasia. In mild dysplasia, the majority of cases (67%) exhibited weak HSP70 staining (intensity 1). In contrast, moderate dysplasia showed predominantly moderate HSP70 staining (60% with intensity 2), and severe dysplasia demonstrated strong HSP70 staining in all cases (100% with intensity 3). The correlation between HSP70 staining intensity and dysplasia severity was statistically significant ($p < 0.01$).

The chi-square test and Fisher's exact test confirmed the significant association between HSP70 expression and the severity of epithelial dysplasia ($p < 0.01$). These results suggest that HSP70 expression increases with the progression of dysplasia and can serve as a potential biomarker for assessing the severity of epithelial dysplastic changes in oral lesions.

Discussion

The results of this study demonstrate a significant correlation between HSP70 expression and the severity of epithelial dysplasia in oral lesions. Our findings align with previous research suggesting that HSP70 plays a crucial role in the pathogenesis and progression of various cancers, including oral squamous cell carcinoma (OSCC) (1,2).

The observed increase in HSP70 expression with the severity of dysplasia is consistent with the hypothesis that HSP70 contributes to cellular transformation and tumor progression. HSP70 is known to enhance cell survival under stress conditions by inhibiting apoptotic pathways and stabilizing oncoproteins (3). This anti-apoptotic function of HSP70 may facilitate the survival of dysplastic cells, allowing for the accumulation of genetic mutations that drive malignant transformation (4).

Our study found that HSP70 was expressed in 80% of mild dysplasia cases, 90% of moderate dysplasia cases, and 100% of severe dysplasia cases. This trend suggests that HSP70 could serve as a valuable biomarker for early detection and grading of dysplastic lesions. Similar findings have been reported in other studies, where increased HSP70 expression was associated with higher grades of dysplasia and poorer clinical outcomes (5,6).

The use of immunohistochemistry (IHC) in this study provided a robust method for detecting and quantifying HSP70 expression in tissue samples. IHC has been widely utilized in cancer research to evaluate protein expression and localization, offering insights into the molecular mechanisms underlying disease progression (7-10). The significant association between HSP70 expression and dysplasia severity observed in our study underscores the potential of HSP70 as a prognostic marker.

However, there are limitations to this study that should be addressed. The sample size was relatively small, and larger studies are needed to validate our findings. Additionally, while our results suggest a correlation between HSP70 expression and dysplasia severity, further research is required to elucidate the underlying mechanisms driving HSP70 upregulation in dysplastic cells.

Future studies could explore the role of HSP70 in promoting dysplastic cell survival and proliferation, as well as its potential as a therapeutic target. Inhibitors of HSP70 have shown promise in preclinical studies, suggesting that targeting this protein could disrupt the survival mechanisms of cancer cells and enhance the efficacy of conventional therapies (8).

Conclusion

In conclusion, our study demonstrates that HSP70 expression is significantly associated with the severity of epithelial dysplasia in oral lesions. HSP70 could serve as a valuable biomarker for assessing dysplastic progression and identifying patients at higher risk of malignant transformation. These findings contribute to the growing body of evidence supporting the role of HSP70 in cancer biology and highlight the potential for developing HSP70-targeted therapies in the future.

References

1. Calderwood SK, Gong J. Heat shock proteins promote cancer: It's a protection racket. Trends Biochem Sci. 2016;41(4):311-23.
2. Murphy ME. The HSP70 family and cancer. Carcinogenesis. 2013;34(6):1181-8.
3. Daugaard M, Rohde M, Jäättelä M. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS Lett. 2007;581(19):3702-10.
4. Mosser DD, Morimoto RI. Molecular chaperones and the stress of oncogenesis. Oncogene. 2004;23(16):2907-18.
5. Yang X, Wang J, Liu C, Grizzle WE, Yu S, Zhang S, et al. Heat shock protein 70 promotes the proliferation of normal and dysplastic cells in human oral mucosa. Cell Stress Chaperones. 2008;13(1):81-92.
6. Zhuang Z, Dai J, Cui L, Liang C, Peng Z, Liu A. The prognostic role of HSP70 in head and neck cancer: A systematic review and meta-analysis. Cancer Cell Int. 2019;19:190.
7. Ramos-Vara JA. Technical aspects of immunohistochemistry. Vet Pathol. 2005;42(4):405-26.
8. Rathore A, Tiwari A, Nazim M, Gupta AK, Gande M, Krishnakumar J. Detection of human papillomavirus and its association with potentially malignant disorders and oral squamous cell carcinoma: A retrospective study. Journal of Pharmacy and Bioallied Sciences. 2022 Jul 1;14(Suppl 1):S820-4.
9. Tiwari A, Ghosh A, Agrawal PK, Reddy A, Singla D, Mehta DN, Girdhar G, Paiwal K. Artificial intelligence in oral health surveillance among under-served communities. Bioinformation. 2023;19(13):1329.
10. Powers MV, Workman P. Inhibitors of the heat shock response: Biology and pharmacology. FEBS Lett. 2007;581(19):3758-69.