ORIGINAL ARTICLE

Estimation of Serum Levels of Heat Shock Proteins (HSP) – 70 in Patients with Oral Dysplastic Lesions of Leukoplakia

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Abstract

Background: *This study was done to estimate the serum levels of Heat shock proteins (HSP) – 70 in patients with oral dysplastic lesions of leukoplakia.* ***Methods:*** *87 patients having clinically leukoplakia and histologically proved dysplasia were taken for the study. The histological dysplastic grades were based on WHO classification of the dysplasia. Only patients with moderate and severe dysplasia were considered for the study. Serum HSP -70 levels were assessed by colorimetric sandwich ELISA test according to the manufacturer's instructions.* ***Results:*** *On comparison of the controls and dysplasia patients HSP-70 levels in serum samples, it was found that the difference was statistically significant.* ***Conclusion:*** *In the present study, HSP-70 activity was significantly higher in oral dysplastic (Leukoplakia) group than in the control group. Further studies with larger sample size and with distribution of different grades of dysplasia are required to substantiate these results.*

Keywords*: Dysplasia, Heat Shock Proteins, Leukoplakia*

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Introduction

Dysplasia is a Greek word meaning abnormal atypical proliferation of tissues. Term dysplasia was introduced by Reagon in 1958 in relation to cells exfoliated from the uterine cervix. It is encountered principally in the epithelia. It is a forerunner of cancer. In past, epithelial dysplasia, epithelial atypia and dyskeratosis were used synonymously. Pindborg (1977) defined epithelial dysplasia as the term used for a lesion in which part of thickness of the epithelium is replaced by cells showing varying degree of atypia. Kumar (1992) defined dysplasia as a disturbance in maturational sequence of stratified squamous epithelium and disturbance in cell kinetics of the proliferative compartment with cytological changes. Paul Freedmen & Stanley Kerpel (1995) define it is the diagnostic term used to describe the histopathological changes seen in chronic, progressive and premalignant disorders of oral mucosa.[1]

OSCC is commonly preceded by a range of tissue and cellular alterations consistent with carcinoma, yet restricted to the surface epithelial layer, termed oral epithelial dysplasia (OED). These changes often manifest in a clinical mucosal lesion. Various attempts have been made to uniformly diagnose and discretely categorize the continuous scale of tissue changes that is OED.[2-5]Tumor markers are substances that are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign (noncancerous) conditions that can aid in the diagnosis of cancer and in the assessment of tumor burden. Tumor markers can often be detected in higher than normal amounts in the blood, urine, or body tissues of some patients with certain types of cancer. Measurements of tumor marker level can be useful-when used along with radiographs or other tests-in the detection and diagnosis of some types of cancer.[6] Heat-shock proteins

(HSPs) are a family of ubiquitous molecular chaperones essential for protein folding and transport within the cell, with HSP - 70 being one of the most abundant proteins in the eukaryotic cell. It participates in structural maturation and conformational regulation of a number of signaling molecules and transcription factors and constitutes up to 1–2% of the cellular protein under physiological conditions. Its expression is several fold enhanced in response to stresses placed upon the cell, e.g. in the context of ultraviolet radiation, heavy metals, malignancies, and inflammation. While Hsp-70 resides primarily in the cytoplasm, it can also be released to extracellular compartments in response to such stressful conditions or upon cell death.[7,8] This study was done to estimate the serum levels of Heat shock proteins (HSP) – 70 in patients with oral dysplastic lesions of leukoplakia.

Materials and Methods

A total of 87 patients having clinically leukoplakia and histologically proved dysplasia were taken for the study. The Study was carried out at Meghna Institute of Dental Sciences Nizamabad. Permission from the Institutional Ethical Committee was obtained before starting the study. Informed consent was obtained from patients to participate in this study. All the clinical types of leukoplakia having dysplasia histologically were considered for the study. (fig. 1) The histological dysplastic grades were based on WHO classification of the dysplasia. Only patients with moderate and severe dysplasia were considered for the study. (fig. 2) The patients were of the age group 24 to 68 years. Out of 87 patients, 49 were males and 38 were females.

The serum samples of the patients were obtained after the diagnosis of the patients were made and the patients who were not received treatment previously. Age and sex matched healthy individuals were taken as controls. All the controls were free of infections and were not suffering from any autoimmune disease. Serum HSP -70 levels were assessed by colorimetric sandwich ELISA test according to the manufacturer's instructions. All the readings were noted and arranged in a tabular form. The results were analyzed using IBM SPSS version 20 with the help of student t test.

Figure 1: Clinical picture of patient with erythroleukoplakia. Histologically this patient was shown to have moderate degree of dysplasia.

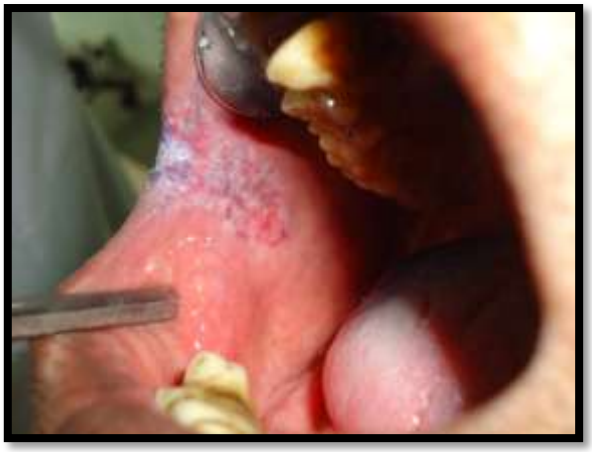
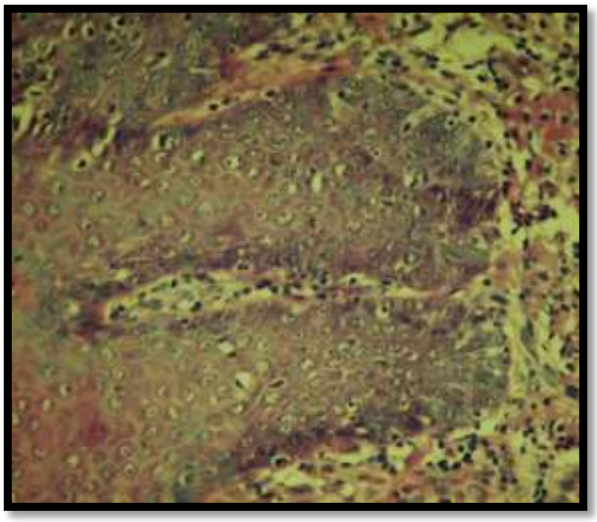


Figure 2: Histological picture of the moderate dysplasia.



Results

A Total of 87 patients were taken for the study. They were divided in two groups Test group which comprised of Diagnosed Patients with Leucoplakia Patients were of the age group 24 to 68 years and out of 87, 49 were males and 38 were females. Control Group was taken to match with the test group.

On comparison of the controls and dysplasia patients HSP -70 levels in serum samples, it was found that the difference was statistically significant. (Student t test, $p<0.01$) (Table 1].

Table 1: Comparison of the HSP- 70 serum levels in controls and patients

Group	Number of patients	HSP -70 levels (Mean ± SD)	t value	p value
Dysplasia patients	87	2.6 ± 0.611	2.5862	<0.01*
Controls	87	1.95 ± 0.508		

Discussion

Many oral cancers are preceded by precancerous lesions which have varied presentations in the oral cavity. In a World Health Organization Workshop held in 2005, these oral lesions with predisposition to malignant transformation were renamed as 'potentially malignant disorders' and this term includes both precancerous lesions as well as precancerous conditions. These potentially malignant disorders are characterized histologically by various degree of epithelial dysplasia. The term 'epithelial dysplasia' is assigned to histopathological changes associated with an increased risk of malignant development.[9,10] Oral Epithelial Dysplasia (OED) is the diagnostic term used to describe the histopathologic changes seen in a chronic, progressive and premalignant disorder of the oral mucosa. OED is not associated with any specific clinical appearance, however, leukoplakia and erythroplakia are the lesions classically associated with dysplastic changes. It is also consistently seen in the mucosa adjacent to the tumor in patients with invasive squamous cell carcinoma. Dysplastic epithelium is found in 25 % of biopsy samples of the leukoplakia.[1]
Histopathology is recognized as the gold standard in the diagnosis of many mucosal diseases and indeed for many other tissue and disease types. In the case of OED, histopathology is also used as a clinical tool to predict the risk of malignant transformation and often guides clinical management and treatment of patients and their mucosal lesions, with clinicians often monitoring milder cases of OED and actively treating through surgical excision those deemed to be severe.12,13 However, the use of histopathology for the diagnosis and categorization of OED has long been considered imprecise, with poor inter and intra-observer agreement and low levels of reproducibility.[4,11]
Tumor markers are a major part of the secondary prevention (detection) efforts. There are major logistic and economic constraints which could be overcome if a simple laboratory test could be devised that would (based on a sample of blood or urine) indicate the presence of cancer-with a high degree of specificity and sensitivity-before there is metastasis.[6] Oral squamous cell carcinoma is one of the most common malignancies worldwide. The individual steps in the etiopathogenesis of OSCC are not clearly identified. Carcinogenesis is a complex process which is controlled by different kinds of genes and mechanisms. Many of the oncogenes including Ras, myc, c-erbB1; antiapoptotic proteins such as Bcl-x and Bcl-2; and several tumor suppressor genes have been recognized in pathogenesis of oral carcinomas. Also, precancerous lesions such as leukoplakias and erythroplakias play a role in oral carcinogenesis. Heat shock proteins (HSPs) form the most ancient defense system in all living organisms. They are a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stresses, including infection, irradiation, heavy metals, ethanol and oxidants. These proteins can be divided into different families according to their molecular weight such as HSP27 (27KDa), HSP70 (70KDa), HSP60 (60 KDa), and HSP90 (90KDa). However HSPs are beneficial to the normal cells. Cancer cells can also use HSPs in response to stress, leading to increased expression. HSPs are detected in neoplasms arising from many tissues and organs such as prostate, adrenal gland, bladder and oral carcinomas. Also recent studies have revealed that HSPs are expressed in cardiovascular diseases.[12-17]

Conclusion

In the present study, HSP70 activity was significantly higher in oral dysplastic (leukoplakia) group than in the control group. This supports the critical role of HSP70 in the development of human oral cancer. Hence, it is

concluded that increased HSP70 could be an objective marker for the presence of epithelial dysplasia. Further studies with larger sample size and with distribution of different grades of dysplasia are required to substantiate these results.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

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