EÜ Dişhek Fak Derg 2017; 38_3: 176-181

Oral Lökoplaki Ve Oral Skuamoz Hücreli Karsinomada Ghrelin Seviyelerinin Değerlendirilmesi: İmmünhistokimyasal Çalışma

The Evaluation Of Ghrelin Expression In Oral Leukoplakia And Oral Squamous Cell Carcinoma: An Immunohistochemical Study

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ÖZET

AMAÇ: Biobelirteçler normal dokuda, prekanseröz lezyonlarda ve kanserli dokuda farklı miktarlarda bulunabilen proteinler veya genlerdir, biobelirteçlerin değerlendirilmesi daha etkin bir şekilde malign transformasyon öngörüsü yapılmasını sağlayabilir. Bu çalışmanın amacı normal oral epitel, oral lökoplaki ve oral skuamoz hücreli karsinomada (OSHK) ghrelin seviyelerinin araştırılarak karşılaştırılmasıdır.

YÖNTEM: Patoloji bölümü arşivinden elde edilen toplam 55 adet daha önce tanısı konulmuş normal oral mukoza (n = 15), oral lökoplaki (n = 18) ve OSHK (n = 22) bloklarından deparafinize edilerek elde edilen preparatlar özel antikorlar kullanılarak immünhistokimyasal olarak boyanmış ve ghrelin seviyeleri değerlendirilmiştir. İmmünhistokimyasal boyamada ghrelin mevcudiyeti mikroskop altında 100 hücre değerlendirilerek sayısallaştırılmıştır. Ghrelin pozitif hücrelerde kahverengi sitoplazmik boyama görülmüştür.

BULGULAR: Normal oral mukozada hücrelerinin % 64'ünde, oral lökoplaki hücrelerinin % 66'sında ghrelin pozitif boyama görülürken, OSHK'da bu boyama yalnızca hücrelerin % 8'inde olmuştur. Diğer gruplar ile karşılaştırıldığı zaman OSHK grubunda ghrelin pozitif boyalı hücre miktarının istatistiksel olarak anlamlı şekilde az olduğu görülmüştür ($P < 0.05$).

SONUÇ: Normal oral mukoza ve oral lökoplaki ile karşılaştırıldığı zaman OSHK grubunda ghrelin seviyesinin daha az olduğu tespit edilmiştir. Oral lökoplaki ve normal oral mukoza gruplarının ghrelinin seviyelerinin benzer olduğu belirlenmiştir. Oral karsinogenezis ile birlikte oluşan ghrelin seviyesinde ki değişikliklerin artmış malign potansiyelin belirlenmesinde bir biobelirteç olabileceği düşünülmektedir.

Anahtar Kelimeler: Ghrelin, oral lökoplaki, oral skuamoz hücreli karsinoma

ABSTRACT

INTRODUCTION*: Biomarkers are proteins or genes that can be differentially expressed in cancer, pre-malign, and normal tissue and the use of biomarkers may help to improve prediction of cancer transformation. The aim of this study was to investigate whether ghrelin is differently expressed in normal oral epithelium, oral leukoplakia, and oral squamous cell carcinoma (OSCC).*

METHODS*: Preparations of deparaffinized blocks obtained from the pathology archives of 55 previously diagnosed cases of normal oral mucosa (n = 15), oral leukoplakia (n = 18), and OSCC (n = 22) were stained immunohistochemically with specific antibodies to evaluate ghrelin expression. The ghrelin expression on immunohistochemical staining was quantified visually by counting 100 cells under a microscopic. Ghrelin-positive cells showed brown cytoplasmic staining.*

RESULTS*: Ghrelin was expressed in 64% of normal oral mucosa, 66% of oral leukoplakia, but in only 8% of OSCC. Compared with the other two groups, the mean ghrelin expression decreased significantly ($P < 0.05$) in the OSCC group.*

CONCLUSION*: Ghrelin expression is decreased in OSCC compared with normal oral mucosa and oral leukoplakia. The ghrelin levels were similar in oral leukoplakia and normal oral mucosa. Ghrelin expression changes with oral carcinogenesis may be a biomarker for determining increased malignant potential.*

Keywords*: Ghrelin, oral leukoplakia, oral squamous cell carcinoma*

INTRODUCTION

Cancer is a major cause of morbidity and mortality, and squamous cell carcinoma accounts for the most cases of oral malignancy.[1] Oral squamous cell carcinoma (OSCC) often develops from premalignant lesions, such as leukoplakia. Leukoplakia has been defined as "white plaques of questionable risk, having excluded (other) known diseases or disorders that carry no increased risk for oral cancer".[2] Histopathologically, leukoplakia may show hyperkeratosis or hyperkeratosis with dysplasia or carcinoma.[3]

Başvuru Tarihi: 12.07.2017
Kabul Tarihi: 18.12.2017

Although dysplasia is the most important predictor of malignant potential, an evaluation of epithelial dysplasia involves substantial subjectivity, and hyperkeratosis without dysplasia may not completely exclude carcinoma or its potential.[4,5,6,7] Hyperkeratotic lesions without dysplasia can also show malignant transformation.[8] The ability of current clinical and histological methods to predict premalignant lesions that will undergo malignant transformation is limited, and it is important to develop other methods for predicting the malignant potential of lesions so that they can be treated appropriately.

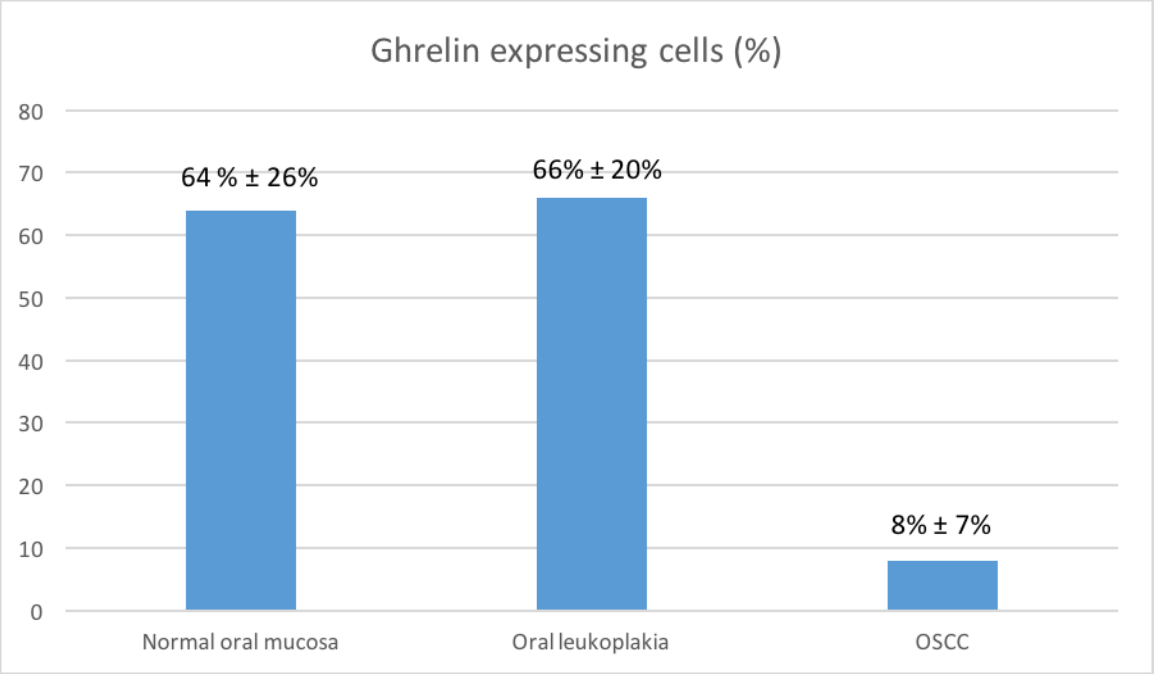

Fig 1: Proportion of ghrelin expressing cells. The ghrelin levels in OSCC were significantly lower than those in oral mucosa and oral leukoplakia (P = 0.001).

Ghrelin is a 28-amino-acid peptide hormone that is produced mainly by the stomach and secreted into the blood.[9,10,11] Ghrelin receptors are growth hormone secretagogue receptors. Ghrelin, which is present in the tissue and circulation in two major forms, des-acylated and acylated ghrelin, plays roles in growth hormone release, regulation of metabolism and appetite, modulation of the immune system and bone metabolism, and stimulation of adipogenesis.[12,13]

Ghrelin and its receptors are expressed by a range of tumor types.[14] Ghrelin regulates events associated with cancer, including cell proliferation and migration, angiogenesis, invasion, and apoptosis. Although much information has been obtained, the role of ghrelin in carcinogenesis and cancer is not fully understood.[14,15,16] To clarify this issue, several studies have compared ghrelin expression in malignant and normal or benign tissues and showed that ghrelin expression may be up- or down-regulated in cancer. It is thought that this difference might constitute a diagnostic tool to differentiate carcinoma from normal tissue.[14,17,18] Although the pathophysiological significance of this expression is unclear, there is evidence that ghrelin could be a useful prognostic or diagnostic marker for recognizing malignant tissue.

Ghrelin is produced by the oral epithelium, fibroblasts, salivary glands, oral keratinocytes, teeth, and OSCC in the oral cavity.[10,19–22] A major challenge is to determine which oral premalignant lesions will transform into carcinoma. Therefore, this study assessed the expression of ghrelin in normal oral mucosa, oral leukoplakia, and OSCC by immunohistochemistry using formalin-fixed paraffin-embedded tissues. The detection of molecular changes associated with the initiation and progression of oral leukoplakia and OSCC could provide new diagnostic and prevention methods and new targets for molecular therapy.

METHODS

This study was approved by the University Ethics Committee (protocol: 01.15.2015-12). Formalin-fixed paraffin-embedded blocks of normal oral mucosa, oral leukoplakia, and OSCC were obtained from the archives

of the Department of Pathology. The 55 samples included 15 samples of normal oral mucosa, 18 samples of oral leukoplakia, and 22 samples of OSCC. The histopathological and clinical information was obtained from the case files. All tissue samples were stained with hematoxylin/eosin and re-examined to confirm the diagnosis according to the World Health Organization 2005 guidelines. Microscopically, all the oral leukoplakia showed hyperkeratosis without dysplasia. The normal oral mucosa cases chosen had no history of oral premalignancy or malignancy. Immunohistochemical staining was performed with a commercial monoclonal ghrelin mouse antibody (ab189162, Abcam, Cambridge, U.K.) diluted 1:100.

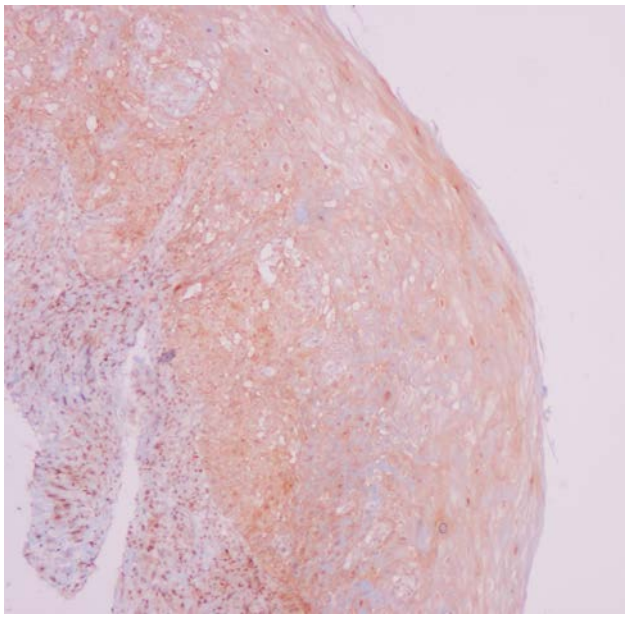

Fig 2A

Immunohistochemistry procedure

Three-micrometer sections were cut from paraffin-embedded blocks and placed on microscope slides. Ghrelin immunohistochemistry was performed on all slides using the specific antibody with the BenchMark XT automated staining system (Ventana Medical Systems/Roche, Tucson, AZ). Immunohistochemical staining was performed using an ultraView Universal DAB detection kit (760-500; Ventana Medical Systems/Roche).

Microscopic Evaluation

The immunostaining was reviewed by two independent evaluators (R.E., F.G.). The ghrelin expression was detected throughout the cytoplasm of oral mucosa, oral leukoplakia, and OSCC specimens. The ghrelin expression on immunohistochemical staining was quantified visually by counting 100 cells under a microscopic at ×40 magnification. Ghrelin-positive cells showed brown cytoplasmic staining, whereas ghrelin-negative cells were stained only with hematoxylin eosin. Any intensity of DAB-positive brown cytoplasmic staining was counted as positive for ghrelin, and the results are presented as a percentage of all cells examined.

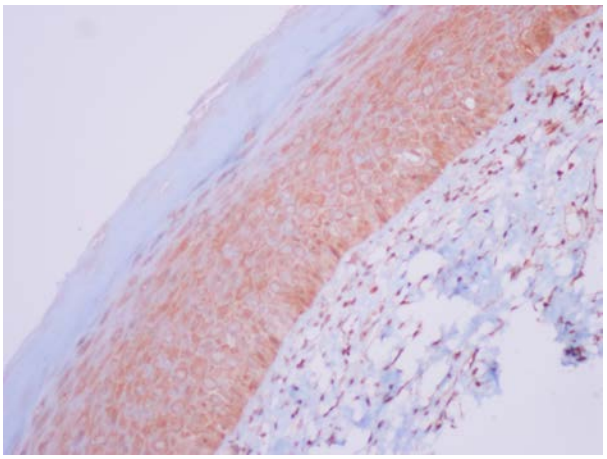

Fig 2B

Statistical Analysis

The ghrelin percentage for each sample was entered into the Statistical Package for the Social Sciences ver. 22 (SPSS, Chicago, IL, USA). The mean ghrelin percentage was calculated for each group and compared with the Mann–Whitney *U*-test and Kruskal–Wallis test. *P*-values < 0.05 were considered to be statistically significant.

RESULTS

The ghrelin expression was evaluated in the cytoplasm of normal oral mucosa, oral leukoplakia, and OSCC cells. Ghrelin was abundant in the cytoplasm of oral mucosa and oral leukoplakia cells, but it was less so in OSCC. Fig. 1 summarizes the ghrelin expression in all of the specimens examined. Fig. 2 shows examples of ghrelin expression (brown) in oral mucosa, oral leukoplakia, and OSCC. The ghrelin levels in OSCC were significantly lower than those in oral mucosa and oral leukoplakia (P = 0.001), whereas the oral leukoplakia and normal oral mucosa ghrelin values were not significantly different.

DISCUSSION

Biomarkers are proteins or genes that are differentially expressed in normal tissue, premalignant lesions, and cancer, and this difference may help to predict the clinical outcome.[23] OSCC carcinogenesis is a multistep process involving biomolecular changes, premalignant lesions, and invasive cancer.[7,24] When the biomolecular

changes begin in tissue, it is possible that the earliest mutations cause only increased keratin formation without visible dysplasia.[3] It is necessary to detect these biomolecular changes at the earliest time to prevent progression of the disease. However, early detection of these changes is impossible, due to the lack of adequate early predictive biomarkers. Numerous studies have compared the biological properties of premalignant lesions with their malignant potential; to this end, the expression of some biomarkers in oral precancerous and cancerous lesions have been compared.[6,23,25–28] The molecular events that cause a premalignant lesion to turn into a carcinoma are still unknown, and it is still impossible to predict which premalignant lesion will progress to carcinoma.[29]

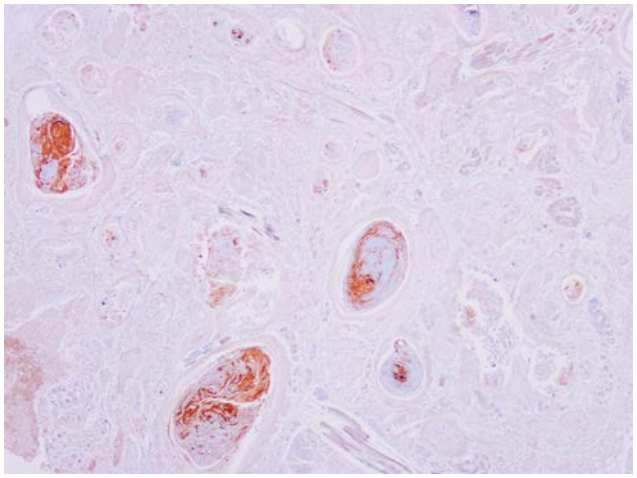

Fig 2C

Studies have evaluated the use of ghrelin as a tumor marker. Karaoğlu et al.[17] found lower ghrelin levels in thyroid papillary carcinomas compared with normal thyroid tissue and suggested that the difference in ghrelin levels constituted a diagnostic tool. Regarding the possible use of ghrelin as a tumor marker, some tumors expressing ghrelin show increased ghrelin levels compared with normal tissue (*e.g.*, breast, colorectal adenocarcinoma, and esophageal SCC), whereas others show decreased levels (*e.g.*, endometrial carcinomas, renal cancer, and salivary gland mucoepidermoid cancer).[17,30–35] The ghrelin expression patterns of various tumors have shown heterogeneous results, and the over- or underexpression of biomarkers compared with healthy tissue added predictive value over microscopic evaluation.[14] The differences in ghrelin expression in different tumors may be related to tissue differences and their embryological origins.

There are two reports on ghrelin expression in OSCC. Alnema et al.[19] investigated OSCC immune-histochemically and found decreased ghrelin expression in cancer compared with benign tissue; they suggested that ghrelin could help distinguish malignant tissue from benign tumors. They also found that ghrelin was expressed in the supra-basal layer of the normal oral mucosa in nonmalignant epithelium adjacent to OSCC samples, whereas ghrelin was expressed in the entire mucosal layer, and especially in keratin pearls, in OSCC.[19] These findings concur with our results. We found that the ghrelin expression level in OSCC was lower than those in oral epithelial tissue and leukoplakia; moreover, the ghrelin expression in OSCC was found especially in keratin pearls. In the second study, Kraus et al.[15] found increased expression of ghrelin and its receptors in OSCC compared with healthy gingiva and benign tissue. The studies evaluating OSCC ghrelin levels yielded conflicting results. Whereas one study found increased ghrelin levels in OSCC, the other two studies, including this one, found decreased ghrelin levels. Ghrelin expression is associated with tumor invasion and metastases.[16] The invasiveness of the carcinomas examined in these studies may have differed, and this difference may have caused the different levels of ghrelin expression.

Only one study has compared the ghrelin level of leukoplakia with those of healthy tissue and OSCC. Kraus et al.[15] found 10-fold elevated ghrelin mRNA and ghrelin levels in leukoplakia compared with healthy gingiva, whereas the ghrelin levels of oral leukoplakia and OSCC were similar. This contrasts with our finding that the oral leukoplakia ghrelin level was similar to that of normal oral mucosa and eight-fold more than that in OSCC. This difference may be explained by the histological diagnosis of the leukoplakia used in these studies. In our samples, all the oral leukoplakia showed hyperkeratosis without dysplasia. The histologic diagnosis of oral leukoplakia was not mentioned in Kraus et al.[15] It is possible that the ghrelin level begins to change with dysplastic changes in oral leukoplakia.

Recently, it is stated inflammatory conditions of oral mucosa is one of the biggest challenges to distunguish bening lesions from potentially malignant lesions, and dysplastic/malignant ones from inflammatory conditions. For this purpose, the patients with chronic oral inflammatory diseases such as periodontitis must be differentiated from those with oral premalignant disorders or oral cancers.[36] The limitation of this study

is the lack of data about the inflammatory condition of study subjects.

CONCLUSION

Some biomarkers have been shown to have statistically significant ability to predict malignant transformation in oral dysplasia.[23] The role of ghrelin in oral carcinogenesis is not fully understood, but the ghrelin expression changes in oral carcinogenesis may be a biomarker that can improve the identification of increased malignant potential. Further studies using larger sample sizes will determine the value of ghrelin as a potential biomarker for oral carcinogenesis.

Acknowledgements: This work was supported by the Scientific Research Project Fund of Cumhuriyet University under project number DİŞ - 156.

Disclosure: None of the authors have any financial interest either directly or indirectly in any company or any of the products mentioned in this manuscript.

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