RESEARCH ARTICLE

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Evaluation of Langerhans Cell Density in Oral Lichen Planus and Squamous Cell Carcinoma

ABSTRACT

Objective: Oral mucosa exposed to many external factors. It has been suggested that oral pathologies may be related to cell-mediated immunity. Dendritic cells are antigen presenting cells that initiate adaptive cellular response. Langerhans cells(LCs), an important member of this group. Oral lichen planus(OLP) is a common chronic mucocutaneous inflammatory disease of unknown etiology. The most common malignancy in the oral cavity is oral squamous cell carcinoma(OSCC). In this study, we aimed to understand the role of LCs in OLP and OSCC which has an important role in mucosal defense.

Methods: A total of 74 biopsies taken from Dentistry Department between 2013-2016 were included into the study. The 74 cases;36 OLP, 28 OSCC and 10 normal mucosa as a control group were retrospectively re-evaluated. After selecting appropriate blocks, to evaluate Langerhans cells Langerin was applied immunohistochemically. Basal and suprabasal located immune positive Langerhans cells were counted in 1mm^2 areas in each case.

Results: In our study, female (60%) predominancy in OLPs, male predominancy(58%) in OSCCs were reported. Age distribution mean was 53±12 in OLPs and 61±21 in OSCCs. OLP was localized in the buccal mucosa in 86% of patients, whereas this rate was only 11%in OSCC cases. LCs density was 87(57-105) in the control group, 104(84-143) in OLPs, and 82(48-128) in OSCCs.

Conclusions: LC density was found significantly higher in OLPs compared to control group and OSCCs.In literature, variable results were published. Determining the density of Langerhans cells in these diseases can be a guide in terms of the pathogenesis of the disease and the improvement of treatment options.

Keywords: Oral, Squamous Cell Carcinoma, Lichen Planus, Langerhans Cell

Oral Liken Planus ve Skuamöz Hücreli Karsinom Olgularında Langerhans Hücre Yoğunluğunun Değerlendirilmesi

ÖZET

Amaç: Oral mukoza pek çok etken tarafından hasara uğratılabilmektedir. Oral patolojilerin hücre aracılı immünite ile ilişki olabileceği öne sürülmüş ve bu konuda çok çeşitli araştırmalar yapılmıştır. Dendritik hücreler, adaptif hücresel cevabı başlatan antijen sunucu hücrelerdir. Bu grubun önemli bir üyesi olan Langerhans hücreleri(LH), tüm stratifiye epitellerde özellikle de skuamöz epitelin orta ve üst kısmında yerleşir. Oral liken planus(OLP) sık görülen, etyolojisi net bilinmeyen kronik mukokutanöz inflamatuar bir hastalıktır. Oral kavitede en sık rastlanan malignite oral skuamöz hücreli karsinomdur(OSHK). Çalışmamızda mukozal savunmada önemli rolü olan Langerhans hücrelerinin OLP ve OSHK'da dağılımını inceleyerek lokal immün cevaptaki rolü hakkında fikir sahibi olmayı amaçladık.

Gereç ve Yöntem: 2013-2016 yılları arasında Diş Hekimliği Fakültesi tarafından gönderilen, 36 adet OLP, 28 adet OSHK ve kontrol grubu olarak 10 adet normal mukoza olmak üzere toplam 74 hasta retrospektif olarak yeniden değerlendirilmiş, uygun bloklar seçilerek immunhistokimyasal olarak Langerin antikoru uygulanmıştır. Her olguda 1mm^2'lik alanlarda bazal ve suprabazal yerleşimli LH'leri sayılmıştır.

Bulgular: Çalışmamızda OLP olgularında kadın(%60),OSHK olgularında erkek hakimiyeti(%58) izlendi. Yaş dağılımı OLP'larda 53±12 iken OSHK'da 61±21 idi. OLP hastaların %86'sında yanak mukozasında yerleşimli iken OSHK olgularında bu oran sadece %11'di. Langerhans hücre yoğunluğu kontrol grubunda 87(57-105), OLP'da 104(84-143), OSHK'da 82(48-128)'du.

Sonuç: Çalışmamızda LH'lerinde sayısal olarak OLP'lerde kontrol ve OSHK olgularına göre istatiksel olarak anlamlı artış izlenmiştir. Literatürde bu durumlarla ilgili çeşitli farklı sonuçlara rastlamak mümkündür. Langerhans hücrelerinin; bu hastalıklardaki yoğunluğunun belirlenmesi, hastalığın patogenezi ve tedavi seçeneklerinin geliştirilebilmesi açısından yol gösterici olabilir.

Anahtar Kelimeler: Oral, Skuamöz Hücreli Karsinom, Liken Planus,Langerhans Hücresi

INTRODUCTION

Oral cavity is a gate of human body. The immune system and microbial flora have balanced contribution that helps to maintain homeostasis of the mucosa (1). Many physical traumas, microbiologic factors, poor oral hygiene, caries, prosthesis and other factors such as nicotine chewing, smoking and alcohol consumption may cause damage on oral mucosa (2) The literature have shown probable relation of oral pathologies with cell-mediated immunity (3).

Dendritic cells are antigen presenting cells that initiate adaptive cellular response (4). Langerhans cells (LCs) are an important member of dendritic cell family presenting antigen to T lymphocytes (4,5). They are playing a role in immunologic response as a defender (6) . After being captured by LCs, antigens are transported to lymph nodes and presented to CD4 -T helper lymphocytes. This process is followed by activation of CD8 -T supressor lymphocytes (7). In oral mucosa, LCs are commonly located in the basal and suprabasal layer of the squamous epithelium (8).

Lichen planus is an immune mediated chronic inflammatory disease generally affects oral mucosa followed by skin, genital mucosa, scalp and nails (9). Oral lichen planus (OLP) is a quite common disease of mucocutaneous region, ranging from 0.5 to 2% frequency (10).

The disease is characterized by degeneration of basal keratinocytes. The process of degeneration is thought to be triggered by antigens caused by trauma, infections, restorative materials...etc (9). The antigens are trapped within epidermis by a plexus of interdigitating LCs, followed by presentation of antigens to T lymphocytes (5). Additionally, Langerhans and mast cells are held responsible for local response and induce lymphocyte migration to subepithelial region (4, 9).

OSCC is the most common carcinoma of oral cavity caused by many etiological factors such as smoking, alcohol, viral agents (HPV...etc). It constitutes approximately 95 % of oral malignancies (11).

Although WHO classified OLP as a precancerous lesion, its' potential transformation to OSCC is still controversial (9,12).

LCs are one of the suggested elements of tumor progression via inapproptiate tumor antigen presentation (5).

MATERIAL AND METHODS

The study was performed at Bezmialem Vakıf University Hospital. It was approved by institutional ethical board with permission number: 5/30 (09.03.2016). The 74 patients sent from Dentistry Clinic to our department between January 2013 to January 2016 were included. Paraffin embedded blocks of 74 patients- 36 OLP, 28 OSCC and 10 normal mucosa as a control group were retrieved from the archieves and appropriate blocks were chosen. After reviewing the slides, 3-micron thick slices were cut and immunohistochemistry was performed by automated Ventana Benchmark XT system, using Langerin protein mouse monoclonal antibody (Cell Marque, Clone 12D6). Appropriate positive control sections were included. After applying Langerin immune stain, positive LCs showed cytoplasmic brown colour. The prepared specimens were evaluated by light microscope and images were captured by digital camera (Nikon Eclipse Ci microscope, Japan). In each case immunohistochemically positive for LCs were counted in areas of $1mm^2$ under x40 magnification by two pathologists. The most stained (hot point), basal and suprabasal areas were chosen for manual counting. Moreover age, gender and localisation were evaluated.

Statistical Analyses: Continuous variables are expressed as mean±SD or median (interquartile range) when appropriate. Categorical variables are expressed as percentages. To compare nonparametric continuous variables, the Kruskal Wallis-test was used. To compare categorical variables, the Chi-square-test was used. The Spearman correlation coefficient were used to determine parametric and nonparametric measure of statistical dependence between two variables. A two-tailed p-values of less than 0.05 were considered to indicate statistical significance. The statistical analyses were performed using software (SPSS 15.0, SPSS Inc, Chicago, Ill).

RESULT

In our study, most of the control biopsies (7/10) and OLPs (31/36) were excised from buccal mucosa. OSCCs were especially located on the tongue (20/28). The gender distribution indicated that control and OLP groups were predominantly female, 60% and 72% respectively, whereas only 42% of OSCCs were female. The age median was 46±17 for control group, 53 ± 12 for OLPs and 61 ± 21 in OSCCs. Langerhans cells were predominantly located in basal and parabasal layers in all groups (Figure 1 ,2, 3). LC density was 87 (57-105) in the control group, 104 (84-143) in OLPs, and 82 (48-128) in OSCCs (Table 1).

Table 1. Langerin positive LCs distribution in groups

(n)	Control (n=10)	OLP (n=36)	OSCC (n=28)
LCs/1 mm^2	87 (57-105)	104 (84-143)	82 (48-128)

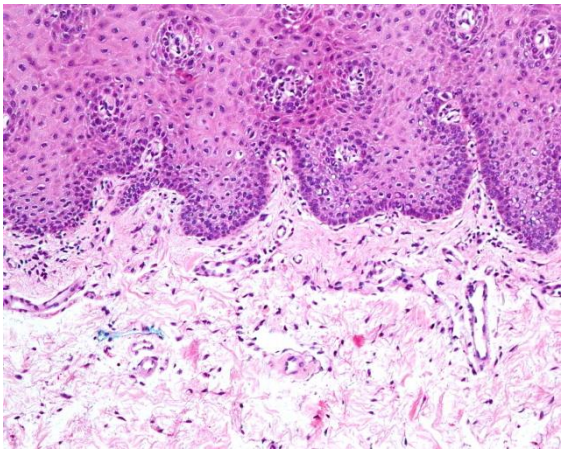

Figure 1a. Control mucosa; Vascularized control tissue from the oral mucosa (HEx100)

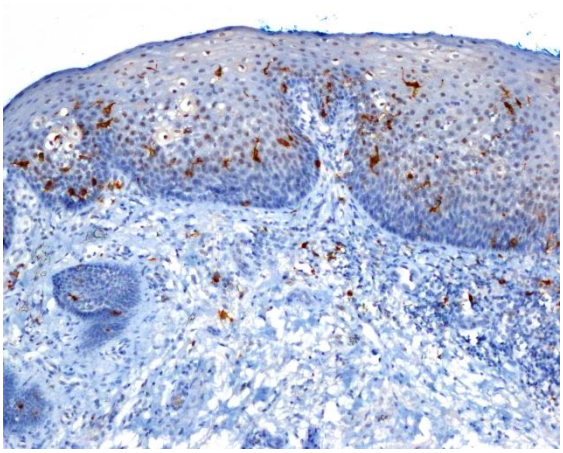

Figure 1b. Control mucosa; Langerin positivity in the control tissue (x100)

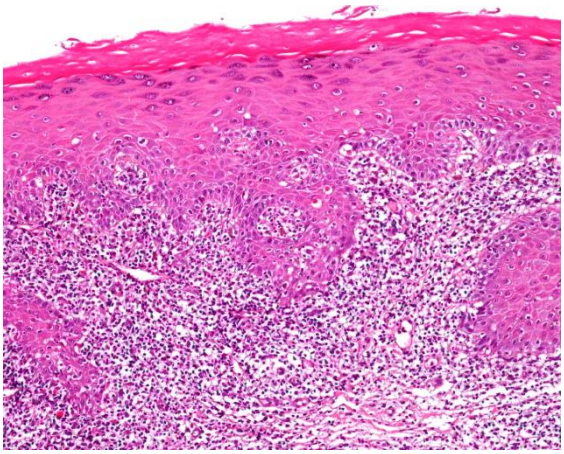

Figure 2a. OLP; Typical histomorphological features of LP (HEx100)

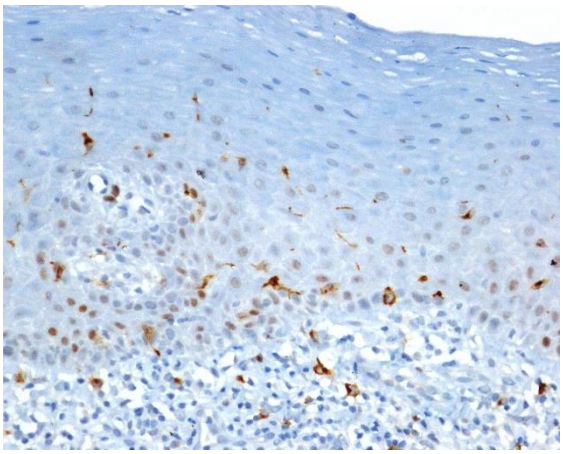

Figure 2b. OLP ; Langerin positivity in OLP (x200)

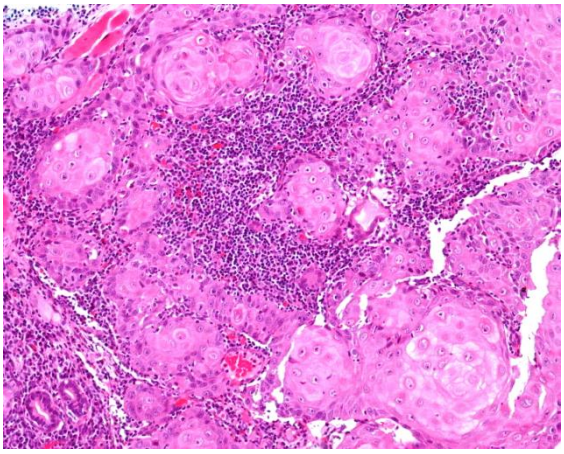

Figure 3a: OSCC; Well-differentiated oral squamous cell carcinoma (HEx200)

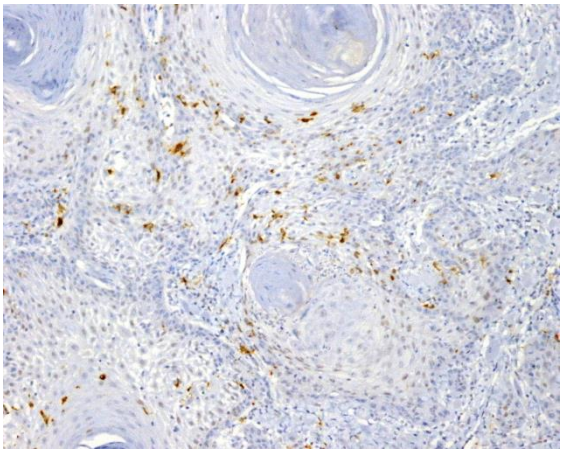

Figure 3b: OSCC; Langerin positivity in well differentiated oral squamous cell carcinoma (x100)

LC density was found significantly higher in OLPs compared to control group and OSCCs. (p = 0.026).

According to tumor differentiation; 11 of the patients were well-differentiated, 7 of them were moderately differentiated, and 5 of them were poorly differentiated. 1 of OSCC was basaloid type and 4 of them could not be graded. We have limited OSCC patients for grade groups, statistically there was no correlation between tumor grade and Langerhans cell density for well-differentiated and poorly differentiated tumors In moderately differentiated OSCCs. LC density was significantly higher than other groups. (p = 0.045).

Table 2. Langerin positive LCs distribution in OSCC groups

(n)	Well (n=11)	Moderately (n=7)	Poor (n=5)
LHS/1 mm^2	64 (48-94)	130 (86-135)	15 (0-128)

DISCUSSION

LCs were first described in 1868, since then many researchers studied their role on immune system and relationship between diseases (5,13). Oral mucosa was the first described part of the body LCs residing (14). In literature addition to their contribution to self tolerance against commensal microoorganisms, their response to inflammatory conditions (Candida, lichen planus, lichenoid drug reactions, human immunodeficiency virus infection and hairy leukoplakia) and anti-tumor immunity were reported (5).

OLP is a common mucocutaneous disorder. Although the exact pathogenesis has not been clarified yet, considerable number of studies associated the disease with cell-mediated immunity (15). OLP is commonly seen in the fifth decade and female/male ratio is approximately 2-3:1 (16). Our findings were consistent with this data.

There were different methods of evaluation of LCs in literature (17). We preferred $1mm^2$ area to evaluate the Langerin positive LCs, because in our opinion it is easy to applicate and take more reliable results.

In our study the number of LCs in OLPs were significantly higher than control group and OSCCs. Our data correlated with the literature as Maloth et al emphasized in their study. They hypothesized the increase may be related to the change in the regulation of locale immune reaction (3). In epithelial damage mostly epithelial-stromal border is affected where keratinocytic apoptosis seen (7). Kumar et al suggested that LCs have pivotal role as taking antigens from apoptotic cells. This adaptive immune response could explain the increase of LCs (17).

As the other carcinomas, OSCCs has multiple steps as following: initiation, promotion and progression. During this steps immunologic factors have important roles. LCs are proposed as one of the factor responsible for host's inadequate presentation of tumor antigens (13). In literature many studies have showed increase of LCs in OSCCs (4). In our study, LC counts were similar to normal mucosa without considering grade of the tumor. Overall OSCCs had no statistically significant difference for Langerin expression. When grouped according to tumor differentiation, moderately differentiated had significantly higher Langerhans cell density than other groups ($p = 0.045$).

Many studies have showed increase of LCs in OSCC (3, 18, 19, 20). One of them was Maloth et al study where LCs were significantly high. Like previous studies they associated the increase with presentation of tumor antigens by LCs (3).

However, gradual decrease in LCs density from well differentiated to poorly differentiated carcinomas was reported. This was explained by immune suppression caused as a result of anaplastic tumor cell induction. Immune suppression has been implicated in the apoptosis of LCs (3).

On contrary there are also studies showing LCs decrease compared to normal mucosa in OSCCs (4, 21). It has been suggested that this situation may occur as a result of accompanying immune suppressive conditions such as smoking, tobacco chewing, alcohol consumption…etc (22). Unfortunately we didn't have detailed history of patients to compare.

CONCLUSION

Immune system plays an important role in the formation, limitation and progression of diseases. LCs have pivotal role in this system. Our findings for LP are compatible with the literature. OSCCs have controversial results for the number of LCs in different studies. The restriction of our study was to have limited number of patients with limited histories. We need more patients with detailed history to gather more reliable data which would help us to show importance of immune response and discover strategies to enhance immune activity for determining prognosis and regulating treatment.

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