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CD1a-Positive Langerhans Cells in Patients with Parotid Gland Malignancies: A Retrospective Clinical Study

Parotis Bezi Kanserli Hastalarda CD1a-Pozitif Langerhans Hücresi: Geriye Dönük Klinik Bir Çalışma

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ABSTRACT

Objective: To determine if Langerhans cell infiltration has any prognostic significance in the malignancy of parotid gland.
Material and Methods: The subjects consisted of 24 patients (12 men, 12 women), aged between 29 and 87 years (mean age, 56±15.51 years), affected by malignancies of the parotid gland and treated surgically. Prognostic factors included disease-free survival, locoregional recurrence, cervical lymph node metastasis, neurovascular invasion, T stage, and distant metastasis. Langerhans cells were identified by immunohistochemical staining for the cell surface marker CD1a, in 24 patients, and the tumor was graded as 0 when there was no cell, as 1+ if there were 1 to 4 cells, and as 2+ when 5 to 9 cells counted. The patological and clinical results of the patients were analyzed statistically.
Results: Although Langerhans cell infiltration was associated with prolonged disease-free survival (mean survival, 24.79±15.69, 35.00±17.32, 34.40 ± 21.80 months in the patients with the density of 0, 1+ and 2+, respectively), there was no statistically significant relationship. There was no relationship between Langerhans cell infiltration and other prognostic criteria either.
Conclusion: Albeit not reaching statistical significance, longer disease free survival was detected in patients with Langerhans cell infiltration. Larger studies should be conducted to more fully elucidate the role of the presence of Langerhans cells at parotid gland malignancy from a prognostic point of view.

Keywords
Prognosis, parotid neoplasms, Langerhans cells, immunity

ÖZET

Amaç: Parotis bezi kanserlerinde Langerhans hücre infiltrasyonunun herhangi bir prognostik bir önemi olup olmadığının belirlenmesi.
Yöntem ve Gereçler: Parotis bezi kanseri tanısı alan ve opere edilen, yaşları 29 ve 87 arasında (ortalama yaş 56±15.51) olan yirmi dört hasta (12 erkek, 12 kadın) çalışmaya alındı. Prognostik faktörler; hastalıksız yaşam süresi, bölgesel rekürrens, servikal lenf nodu metastazı, nörovasküler invazyon, T evresi ve uzak metastaz olarak belirlendi. CD1a hücre yüzey belirleyicisi için yapılan immünohistokimyasal boyama ile yirmi dört hastada Langerhans hücreleri tespit edildi ve hiçbir Langerhans hücresi yoksa 0, 1-4 arası hücre varsa 1+, 5-9 arası hücre tespit edilmiş ise 2+ olarak derecelendirildi. Hastaların patolojik ve klinik sonuçları istatistiksel olarak değerlendirildi.
Bulgular: Langerhans hücre infiltrasyonu, uzamış hastalıksız yaşam süresi ile birliktelik göstermesine rağmen (ortalama yaşam süresi 0, 1+ ve 2+ olarak derecelendirilen hastalarda sırasıyla 24.79±15.69, 35.00±17.32, 34.40±21.80 aydır) istatistiksel açıdan bir ilişki olmadığı belirlendi. Aynı zamanda Langerhans hücre infiltrasyonu ile diğer prognostik kriterler arasında da bir ilişki tespit edilmedi.
Sonuç: Her ne kadar istatistiksel açıdan bir önemi olmadığı belirlenmesine rağmen Langerhans hücre infiltrasyonu tespit edilen hastalarda uzamış hastalıksız yaşam süresi olduğu görülmüştür. Parotis bezi kanserlerinde, Langerhans hücre varlığının prognostik önem açısından rolünün daha çok açıklanabilmesi için daha fazla sayıda hasta içeren çalışmalar yapılmalıdır.

Anahtar Sözcükler
Prognoz, parotis tümorü, Langerhans hücreleri, immünite

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INTRODUCTION

It has been established that the immune system is active in preventing cancer growth, and T- lymphocytes play a major role in tumoral immune response.[1,2] However, T cell activation requires particular antigen presenting cells, namely, the macrophages and the dentritic cells found in different lymphoid organs.[3-5] Langerhans cells are dendritic mononuclear cells which have been recognized as the most potent antigen-processing and presenting cells for many types of T-cell responses.[6,7] Originally, LCs reside in the bone marrow and being transferred via the bloodstream to the peripheral tissues. Once any injury or inflammotory reaction takes place they become fully activated and engulf antigenic particles directly. Afterwards, they pass into the regional lymph nodes and process the antigens to be presented to T and B cells. Several clinical studies have shown the presence of a dense Langerhans cell (LC) infiltration in primary tumor specimens including lung, colon, gastric, and uterine cervix.[8-11] These investigations have also demonstrated a better survival rate in the patients with the LC infiltration than those without it. Likewise, there was also evidence that the relationship between the infiltration of LCs and prognosis in head and neck tumors exists. However, few studies have examined the signifiance of LC infiltration in tumors of the parotid gland so far.[12-15]

We investigated the prognostic significance of CD1a-positive LC infiltration in malignancies of the parotid gland and tried to demonstrate its possible relationship with locoregional recurrence, distant metastasis, cervical lymph node metastasis, disease-free survival, neurovascular invasion, and T stage.

MATERIAL AND METHODS

The study group included 24 patients (12 men, 12 women), aged between 29 and 87 years (mean age, 56 ± 15.51 years), affected by malignancy of the parotid gland and treated surgically at the Department of Otolaryngology-Head and Neck Surgery of the Baskent University Faculty of Medicine between 2000 and 2007. The study was approved by the local ethic committe. The patients underwent superficial, total, or radical (total parotidectomy with facial nerve sacrifice) parotidectomy and neck dissection depending on the extent of the lesion. Orbital exentration was done only in one case because the primary tumor was orbital epidermoid carcinoma. The patients who were previously treated in another center and had recurences, those with positive surgical margins and those who developed a second primary tumor or died of unrelated causes during follow-up period were excluded from the study. Prognostic significance was assessed in relation to the cervical lymph node metastasis, disease-free survival, locoregional recurrence, neurovascular invasion, T stage, and distant metastasis. The studied specimens included 4 primary epidermoid carcinomas, 1 metastatic epidermoid carcinoma, 4 adenoid cystic carcinomas, 4 mucoepidermoid carcinomas, 3 carcinoma ex pleomorfic adenomas, 2 adenocarcinomas, 5 lenfomas, 1 acinic cell carcinoma. The period of observation ranged from 12 to 68 months (median follow-up of the group was 28.92 ± 17.29 months).

In order to quantify LC infiltration in parotid gland malignancy, archived hematoxylin and eosin (H&E)-stained slides from each case were reexamined under the light microscope and suitable paraffin blocks were selected for immunohistochemical study. Five micrometer-thick sections were cut from each block. These slides were immunostained with a biotin-streptavidin complex system (AEC+ Substrat chromogene Ready to Use, K3469, DAKO, Denmark) for CD1a (NeoMarkers, MS.1856, R7) with Detect Super Stain System HRP kit (Labs Inc. IDST 1007).

CD1a-positive Langerhans cells were assessed at x400 magnification with light microscope. Since AEC used a color reagent, an orange-brown color staining in cytoplasm and cell membrane was considered to be positive for CD1a-positive Langerhans cells. Five high power fields (x400 magnification) per patient were examined from intratumoral and peritumoral areas and an average number of positively staining cells per field was calculated and scored as 0 when there was no cell, as 1+ if there were 1 to 4 cells, and as 2+ when there were 5 to 9 cells (Figure 1, 2).

The patological and clinical results of the patients were analyzed statistically by Kruskal-Wallis test, Mann-Whitney U-test with Bonferroni-Holm correction and chi-square test in the SPSS program for Windows (13.0, SPSS Inc, Chicago, Ilinois). At chi-square test, Fisher's exact test was used on 2x2 tables when an expected frequency of a cell was less than 5.

RESULTS

All 24 specimens of parotid gland malignacies were stained with the CD1a antibody. Five of specimens

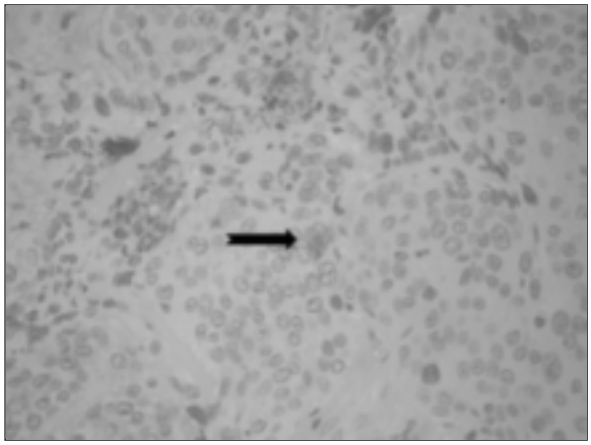

Figure 1. CD1a positive Langerhans cells (arrow) in epidermoid carcinoma of salivary gland which scorred as 1 (CD1a x 400).

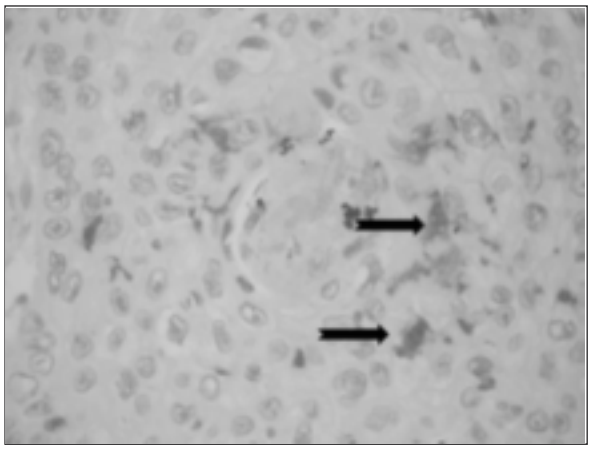

Figure 2. CD1a positive Langerhans cells (Arrow) in epidermoid carcinoma of salivary gland which scorred as 2 (CD1a x 400).

(20.8%) were assessed as 1+ LC infiltration, and another 5 (20.8%) were scored as 2+ LC infiltration. There was no LC infiltration in the rest of specimens (14 of 24, 58.4%). LCs were interspersed within the tumor nest. There was no LCs infiltration in peritumoral tissue.

Total parotidectomy had been performed on 10 patients, and superficial parotidectomy had been performed on 10 patients. Four patients had undergone radical parotidectomy. Ten patients had received postoperative radiation therapy, 4 patients had received chemoradiotherapy and 3 patients had received chemotherapy. The patients had undergone neck dissection when indicated. Five patients (20.8%) had had histopathologically confirmed cervical lymph node metastasis, 8 patients (33.3%) had had neuro-vascular invasion, and two patients (8.3%) had developed distant metastasis during follow-up. There had been only one (4.17%) locoregional recurrence. The T stages of the patients had been as follows; 13 (54.2%) T1, 2 (8.3%) T2, 1 (4.17%) T3, 2 (8.3%) T4a. The patients with lymphoma or metastatic tumor were not included in T classification. Among T1 patients there were 1+ LC infiltration in 2, and 2+ LC infiltration in other 2 patients. Whereas in T2 patients, there were 1+ LC infiltration in 1 and 2+ LC infiltration in 1 patient. No LC infiltration was detected in T3 and T4a patients.

When the relationship between LC infiltration and disease-free survival was analyzed, disease-free survival rates were 24.79 ± 15.69 months in patients without LC infiltration, 35.00 ± 17.32 months in patients with 1+ LC, and 34.40 ± 21.80 months in patients with 2+ LC (Table 1). Although there was an increasing survival in patients with LC infiltration, it did not reach a statistically significance (P_{0-1}= 0.226; P_{0-2}=0.353; P_{1-2} =0.917) (Table 2). Furthermore, there was not statistically significant difference between the LCs infiltration in the primary tumor and the other prognostic criteria (Table 3).

DISCUSSION

Tumor and host immunogenic interaction is important in the prognosis of the head and neck cancer. Lymphocytes, leucocytes, eosinophils, plasma cells, macrophages, and dentritic cells (DCs) have been extensively investigated as a host response against tumor.[9,11,16-18] There seemed to be a general agreement that a better prognosis was associated with the presence of lymphocytes infiltration.[11,19] The majority of tumor infiltrating lymphocytes were shown to be the T cells. LCs are dentritic cells. Functionally, they can present antigens to T cells and are capable of stimulating antigenspesific T cells.[20] Specifically, it was stated that the infiltration of LC and their precursors were correlated well with the infiltration of T- lymphocytes in tumoral

Table 1. The relation between Langerhans cell infiltration and disease-free survival in parotid carcinoma.

Skor	n	Survival (month) mean±SD	P*
0	14	24.79 ± 15.69	0.395
1+	5	35.00 ± 17.32	
2+	5	34.40 ± 21.80	

*Kruskal WallisTest

Table 2. The relation between Langerhans cell infiltration scores and disease-free survival in parotid carcinoma.

Langerhans cell infiltration		n	Survival (month) mean ± SD	P*
Score	0	14	24.79 ± 15.69	0.226
	1+	5	35.00 ± 17.32	
Score	0	14	24.79 ± 15.69	0.353
	2+	5	34.40 ± 21.80	
Score	1+	5	35.00 ± 17.32	0.917
	2+	5	34.40 ± 21.80	

*Mann Whitney U Test

tissue.[21] Ambe et al demonstrated that lymphoctes were densely localized in and around the tumor, whereas LCs were situated very close to tumor cells.[9] They further speculated that LCs, as the antigen presenting cells, might have infiltrated the tumor tissue, recognized the tumor spesific antigens, and stimulated the T cells to infiltrate the the tumor tissue. Therefore, LCs seem to play an important role in cell-mediated immune reaction against neoplastic cells through the recognition and presentation of tumor antigens to T-lymphocytes.

Many subpopulation of Langerhans cells have been demonstrated recently, by immunohistochemical studies and functional differences among them have been delineated.[15,22] Therefore, it is possible that a quantitative increase in the number of these cells may not always be linked to a functional activation of immune responsiveness.[23] In general LCs were determined by means of the reaction with S-100 antigen, but this antigen was shown to be present on other cells as well.[24] At present, CD1a molecule and the positive reaction with this glycoprotein are considered fundamental to the determination of LCs.[25,26] There is paucity of information about the CD1a-positive LCs and prognosis in patients with cancer. However, there is considerable evidence from previous studies, that a marked infiltration of tumors with S100-positive LCs was associated with improved prognosis.[12,13] Interestingly, Goldman et al did not observe an association between S100-positive DCs and outcome in their patients.[14] On the other hand, in their series, they found a positive correlation between the CD1a-positive peritumoral subpopulation of DC and the outcome.

There is an increasing interest to the relationship between LCs and prognosis of the various carcinoma. Improved survival was associated with prominent DC infiltration in lung, colorectal, uterine cervix, and gastric tumors.[8-11] These reports suggested that LC played a significant role in the host defence mechanisms against cancers. Tsujitani et al[10] reported that the survival time of patients with Stage III gastric carcinoma correlated well with the density of LC and on early or far advanced gastric carcinoma did not reveal the same relationship. However, they also stated that there was no relationship between the density of LC and various clinical and morphologic features, including tumor differentiation, depth of invasion, gross appearance, classification of lymph node removal or age and sex. They suggested that in a certain phase of tumor development, immunologic defence mechanisms of the host against tumor might be effective. Nakano et al[11] stated that during and after radiotherapy in cervical cancer, LCs were more important in the defence mechanisms against tumor than other immune system subsets. Relatively few studies have examined head and neck primary tumors. Patients with papillary thyroid carcinoma and those with nasopharyngeal carcinoma have been shown to have improved survival when marked LC infiltration was present.[18,27] Gallo et al[12] found that LCs were interspersed within the tumor nests, and rarely LCs were fo-

Table 3. Reference table for statistical analysis.

Analysis	Statistical test	Significance
Langerhans vs. survival	Kruskal- Wallis	P=0.395
Langerhans vs. survival	Mann-Whitney	P Score 0-1= 0.226
		P Score 0-2= 0.353
		P Score 1-2= 0.917
Langerhans vs. survival	Kaplan Meier	P=0.420
		Long Rank =0.651
Langerhans vs. lymph node metastasis	X2Test (Fisher exact)	P=0.510
Langerhans vs. locoregional recurrence	X2Test (Fisher exact)	P=0.191
Langerhans vs. neurovascular invasion	X2Test (Fisher exact)	P=0.843
Langerhans vs. distant metastasis	X2Test (Fisher exact)	P=0.459
Langerhans vs. T stage	X2Test (Fisher exact)	P=0.258

und around the tumor tissue in 88 patients with laryngeal SCC. Although they found no correlation between the tumor stage and the degree of LC infiltration, low, intermediate, and high densities of LC infiltrations were associated with 5-year survivals of 0%, 62%, and 61%, respectively. Yilmaz et al[13] determined that the increased Langerhans cell infiltration was significantly related to decreased locoregional recurrence, decreased cervical lymph node metastasis, and prolonged disease-free survival in patients with cancer of the larynx. They also suggested that LC infiltration might be determined on a biopsy specimen and it might be useful in deciding whether or not to perform elective neck dissection. It is of note that all the studies above mentioned, have all aimed to determine the prognosis in association with S100-positive LC infiltration.

Although LC infiltration in head and neck cancers were studied by some investigators, little is known of the quantitative assessment and prognostic significance of the LC infiltration in parotid gland cancer (malignancy).[15] Like tumors in other sites, a parotid gland tumor is often characterized by an inflammatory reaction that follows the growth of neoplastic elements. Wischatta et al.[15] demonstrated that mature DCs belonging to interstitial/dermal DC lineage were distributed sparsely in the stroma of parotid gland tumors but LCs were virtually absent within and around the tumors. Although a small sized study, they speculated the reason for the observed paucity of LCs might be the inability of tumor cells to produce CCL20/MIP-3-alpha, the very chemokine that specifically attracts LC precursors. Taking a further step, in their conclusion, they proposed DC vaccination in combination with other modalities for the treatment of parotid gland carcinoma to be taken into consideration.

The purpose of this article was to analyze the relationship between the LC infiltration and the prognosis of the patients with malignant tumors of the parotid gland. Yilmaz et al[13] have studied the presence of LCs both in and around the tumor in larynx cancer and could not reveal a difference. In the study of Goldman,[14] done in tongue cancer, intratumoral CD1a-positive subpopulation of DCs was not associated with improved outcome. They stated that peritumoral DCs were immunologically active, however, the cells that migrated into the tumor might have been somehow blocked or sequestrated by the tumor. On contrary to the findings of Wischatta et al[15] who could not observe LCs within and around the parotid gland tumor, we found that LC infiltration was present within the tumor nest. Although the degree of infiltration was correlating well with disease free survival, it did not reach a statistical significance. There was not any association between other prognostic criteria and degree of LC infiltration. The reason for lack of this association may be the blockage or sequestration of LCs that migrated into the tumor as Goldman et al[14] had stated. This might also explain the disappereance of LC infiltration as the stage and the bulk of the tumor increased. Larger tumor may suppress the migration or survival of LC, decreasing the antitumor immune response and worsening prognosis.[13,14] Small numbers in each subgrup, and different histopathological origins of the tumors might be the culprit for failure to demonstrate a statistically sound evidence for the relationship between the prognostic factors and LCs infiltration. Different histopathologic subtypes may have different biological behaviours and evoke different degree of immunogenic responses. As the salivary gland malignant neoplasm represents about 6% of all head and neck malignancies, the relative rarity of parotid gland cancers makes this study difficult.[28]

In conclusion, larger studies are needed to better delineate spesific functional role of LCs in the immune response in parotid gland tumors. On the other hand although a statistical significance was not achieved, our results suggested a longer disease free survival in patients with parotid gland cancer(malignancy) as the degree of LC infiltration increases in the tumoral tissue.

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REFERENCES

1. Urban JL, Schreiber H. Selection of macrophage resistant progressor tumor variants by the normal host:requirement for concomitant T cell mediated immunity. J Exp Med 1983;157(2):642-56.
2. Shimokawara I, Imamura M, Yamanaka N, Ishii Y, Kikuchi K. Identification of lymphocyte subpopulations in human breast cancer tissue and its significance. Cancer 1982;49(7):1456-64.
3. Koide Y, Awashima F, Yoshida TO, Takenouchi T, Wakisaka A, Moriuchi J, et al. The role of three distinct Ia-like antigen molecules in human T-cell proliferative responses:effect of monoclonal anti-Ia-like antibodies. J Immunol 1982;129: 1061-9.
4. Inaba K, Steinman RM. Accessory cell-T lymphocyte interactions. J Exp Med 1986;163:247-61.
5. Stingl G, Katz SI, Shevach EM, Rosenthal AS, Green I. Analogous functions of macrophages and Langerhans'cells in the initiation of the immune response. J Invest Dermatol 1978;71:59-64.
6. Katz SI, Cooper KD, Iijima M, Tsuchida T. The role of Langerhans cell in antigen presentation. J Invest Dermatol 1985;85:96-8.
7. Nussenzweig MC, Steinman RM, Gutchinov B, Cohn ZA. Dentritic cells and accessory cells for the development of antitrinitrophenyl cytotoxic T lymphocytes. J Exp Med 1980;152:1070-84.
8. Furukawa T, Watanabe S, Kodama T, Sato Y, Shimosato Y, Suemasu K. T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. Cancer 1985;56:2651-6.
9. Ambe K, Mori M, Enjoji M. S-100 protein-positive dentritic cells in colorectal adenocarcinomas. Distribution and relation to the clinical prognosis. Cancer 1989;63:496-503.
10. Tsujitani S, Furukawa T, Tamada R, Okamura T, Yasumoto K, Sugimachi K. Langerhans cells and prognosis in patients with gastric carcinoma. Cancer 1987;59:501-5.
11. Nakano T, Oka K, Takahashi T, Morita S, Arai T. Roles of Langerhans'cells and T-lymphocytes infiltrating cancer tissues in patients treated by radiation therapy for cervical cancer. Cancer 1992;70:2839-44.
12. Gallo O, Libonati GA, Gallina E, Fini-Storchi O, Giannini A, Urso C, et al. Langerhans cells related to prognosis in patients with laryngeal carcinoma. Arch Otolaryngol Head Neck Surg 1991;117:1007-10.
13. Yilmaz T, Gedikoglu G, Celik A, Onerci M, Turan E. Prognostic significance of Langerhans cell infiltration in cancer of the larynx. Otolaryngol Head Neck Surg 2005;132:309-16.
14. Goldman SA, Baker E, Weyant RJ, Clarke MR, Myers JN, Lotze MT. Peritumoral CD1a-positive dentritic cells are associated with improved survival in patients with tongue carcinoma. Arch Otolaryngol Head Neck Surg 1998;124:641-6.
15. Wischatta M, Brunhuber T, Neher A, Kacani L, Romani N, Schrott-Fischer A, et al. Phenotypic characterization and distribution of dentritic cells in parotid gland tumors. ORL J Otorhinolaryngol Relat Spec 2004;66:313-9.
16. Goldsmith MM, Belchis D, Cresson DH, Merritt WD 3rd, Askin FB. The importance of the eosinophil in head and neck cancer. Otolaryngol Head Neck Surg 1992;106:27-33.
17. Höyry P, Tötterman TH. Cytological and functional analysis of inflammatory infiltrates in human malignant tumors. I. Composition of the inflammatory infiltrates. Eur J Immunol 1978;8:866-71.
18. Nomori H, Watanabe S, Nakajima T, Shimosato Y, Kameya T. Histiocytes in nasopharyngeal carcinoma in relation to prognosis. Cancer 1986;57:100-5.
19. Watt AG, House AK. Colonic carcinoma: a quantitative assessment of lymphocyte infiltration at the periphery of colonic tumors related to prognosis. Cancer 1978;41:279-82.
20. Stingl G, Tamaki K, Katz SI. Origin and function of epidermal Langerhans cells. Immunol Rev 1980;53:149-74.
21. Watanabe S, Sato Y, Kodama T, Shimosato Y. Immunohistochemical study with monoclonal antibodies on immune response in human lung cancer. Cancer Res 1983;43:5883-9.
22. Schmitt D, Dezutter-Dambuyant C, Faure M, Thivolet J. Recent advances of ultrastructural immunocytochemistry of epidermal Langerhans' cells. Br J Dermatol 1985;113:2-9.
23. Fox SB, Jones M, Dunnill MS, Gatter KC, Mason DY. Langerhans' cells in human lung tumours:an immunohistological study. Histopathology 1989;14:269-75.
24. Alcalay J, Goldberg LH, Wolf JE Jr, Kripke ML. Variations in the number and morphology of Langerhans' cells in the epidermal component of squamous cell carcinomas. Arch Dermatol 1989;125:917-20.
25. Duan H, Koga T, Masuda T, Mashino T, Imafuku S, Terao H, et al. CD1a+, CD3+, CD4+, CD8+, CD68+ and cutaneous lymphocyte-associated antigen-positive cells in Bowen's disease. Br J Dermatol 2000;143:1211-6.
26. Mazal PR, Hainfellner JA, Preiser J, Czech T, Simonitsch I, Radaszkiewicz T, et al. Langerhans cell histiocytosis of the hypothalamus: diagnostic value of immunohistochemistry. Clin Neuropathol. 1996;15:87-91.
27. Schroder S, Schwarz W, Rehpenning W, Loning T, Bocker W. Dentritic/Langerhans cells and prognosis in patients with papillary thyroid carcinoma:immunohistochemical study of 106 thyroid neoplasms correlated to follow-up data. Am J Clin Pathol 1988;89:295-300.
28. Simental A, Carrau RL. Malignant neoplasms of the salivary gland. In: Cummings CW, Flint PW, Harker LA, Haughey BH, Richardson MA, Robbins KT, Schuller DE, Thomas JR, eds. Cummings Otolaryngology-Head and Neck Surgery, 4th ed. Philadelphia: Mosby Inc; 2005. p. 1378-1405.