CASE REPORTS

Primary malignant non-Hodgkin’s lymphomas of salivary glands

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Abstract
We evaluated the medical record of patients with salivary gland neoplasms diagnosed at Timisoara City Hospital from 2002 to 2009. A study has been carried out for seven years on 204 cases of salivary gland tumors and only two cases of salivary gland lymphomas were diagnosed. The two cases were females of 71- and 49-year-old, respectively. The formalin-fixed paraffin-embedded tissue samples were cut in 4 µm thick sections and stained with Hematoxylin and Eosin. The primary monoclonal antibodies for the immunohistochemical analysis were the followings: LCA (2B11, Dako), CD20 (L26, Dako), cytokeratin (MNF116, Dako), p53 (DQ-7, Dako), and PCNA (PC-10, Dako). The histopathology and immunohistochemistry suggested in the first case a low-grade diffuse large B-cell mucosa associated lymphoid tissue lymphoma and in the second case a high-grade extranodal marginal zone B-cell lymphoma.

Keywords: non-Hodgkin’s lymphomas, salivary gland, parotid gland, mucosa-associated lymphoid tissue lymphoma.

Introduction
Lymphomas primary located in the salivary gland tissue are very rare and constitute about 2–5% of all salivary gland neoplasms. Most cases of lymphoma involve the major salivary glands, frequently the parotid (50–93%) and submandibular glands [1]. These neoplasms may arise from an intraparotid lymph node or in the gland itself. In the normal parotid gland there are intraglandular lymph nodes, that is why it is often difficult to make a distinction between lymphoma arising primarily in the salivary gland and those of the lymph node origin embedded in the salivary gland. If these lymph nodes are affected by a malignant lymphoma and the glandular parenchyma is not, then the lymphoma should be considered as nodal type. The differential diagnosis is not always easy to do because there are cases of lymphomas originated from intraglandular lymph nodes and with extensive parenchyma involvement. We consider a primary salivary gland lymphoma the cases in which the main disease occur here and the parenchyma of the gland is involved. The morphology and prognosis are similar for both origin places [2].

Primary malignant lymphomas of the salivary gland are predominantly B-cell type lymphomas and they include MALT-lymphoma, follicular and diffuse large B-cell lymphoma [2]. Salivary gland T-cell lymphomas are very rare [4]. Anaplastic large cell lymphoma is one of the distinctive subtypes of non-Hodgkin’s lymphoma of the T-cell phenotype [5].

Extranodal NK/T-cell lymphoma of nasal-type can affect in rare occasion the salivary glands. Hodgkin’s lymphoma as a primary salivary gland neoplasm is uncommon – it has been reported both classical Hodgkin’s lymphoma and nodular lymphocyte predominant Hodgkin’s lymphoma, but involving only the parotid gland. In rare cases, Hodgkin’s lymphoma arises from a preexisting Warthin’s tumor [6].

Non-Hodgkin’s lymphoma of salivary gland origin accounts for 5% of all primary extranodal non-Hodgkin’s lymphomas and 2% of all salivary gland tumors [2]. Most cases of non-Hodgkin’s lymphoma arising in salivary glands are of B-cell lineage including low-grade B-cell lymphomas of mucosa-associated lymphoid tissue (MALT), diffuse large B-cell lymphomas and follicular lymphomas [7].

Diffuse large B-cell lymphoma (DLBCL) is an infiltrative tumor, associated with the destruction of the salivary gland parenchyma and interstitial inflammation of the restant salivary acini. The histopathological features exhibit large lymphoid cells, which may resemble centroblasts or immunoblasts and in some cases, this type of lymphoma represent the transformation from a preexisting extranodal marginal B-cell lymphoma of MALT-type [1, 2].

MALT-lymphoma arises in extranodal sites such as the gastrointestinal tract, thyroid and salivary glands [2, 4, 7]. The extranodal marginal zone B-cell lymphoma of MALT-type is the main type of lymphoma affecting primary salivary glands, but cases of follicular lymphoma and mantle cell lymphoma were also described [8, 9].

Extranodal marginal zone B-cell lymphoma of MALT-type (EMZBCL) is an extranodal low-grade lymphoma consisting of small B-cells, centrocyte-like cells and frequently develops in the settings of lymphoepithelial sialadenitis (LESA) in patients with Sjögren’s syndrome [10].
A variable period of time is described between the documented occurrence of LESA and the development of malignant lymphoma. This period may vary from six to 29 years. EMZBCL of salivary gland can show histological transformation to a higher grade. Such cases had been reported in the stomach. Recent data suggest that patients with EMZBCL, which also had LESA, may develop extensive extra salivary gland lymphoma or even large B-cell lymphoma [2].

Mantle cell lymphoma can also involve salivary glands but often reveals other sites of the disease and has a worse prognosis than MALT-lymphomas [2].

Follicular lymphomas interest salivary glands, but for the head and neck region are found predominantly in the lymph node and also in the orbit and thyroid gland; there are studies in which these types have originated in the lungs [11–14].

Material and Methods

Between the years 2002–2009, we evaluated 204 cases of salivary gland tumors – only two cases of lymphoma arising in salivary gland tissue were reported at Timisoara City Hospital.

Patients with evidence of malignant lymphoma elsewhere at the time of diagnosis were excluded.

The lymphomas were located in the right parotid gland, and at clinical examination suggested fixation to the adjacent structures. The cervical lymph nodes were not palpable.

Case No. 1

A.D., a 71-year-old female, presented a tumor mass in the front of the right ear. Grossly the mass was 5/3.5/1.5 cm, soft, with white and gray areas intermingled with adipose tissue and necrosis areas on the cut surface.

Case No. 2

E.V. is a 49-year-old female who presented with a right parotid mass that had been growing over a period of four weeks. In this case, there was a history of benign lymphoepithelial lesions. The patient has been diagnosed with right parotid Mikulicz’ disease in 2001 and the right parotid gland lymphoma diagnose was made seven years later. Grossly, the tumor mass was firm, encapsulated, measured 6.5/6.3/6.5 cm. On the cut surface it had yellowish-tan color with necrosis and hemorrhagic areas.

The tissue samples were fixed in 10% formalin, embedded in paraffin, cut into 4 µm thick sections and stained with Hematoxylin–Eosin (HE).

Immunohistochemical study was performed on the two cases and it had as an aim to complete the histopathologic study and add new data to the clinical and histopathological diagnosis of these two rare tumors. For the immunohistochemical evaluation we have used monoclonal antibodies against LCA (2B11, Dako), CD20 (L26, Dako), cytokeratin (MNF116, Dako), p53 (DQ-7) and PCNA (PC-10, Dako) using EnVision (K5007, Dako) detection system.

Results

Histological examination on HE-stained section of the tumors biopsies showed the following aspects:

- In case no. 1, the tumor was composed of a diffuse proliferation of lymphoid cells with destruction of normal architecture of ducts and acini of the parotid gland. Lymphoepithelial lesions are represented by expanded ducts which are distorted by neoplastic lymphoid cells; the lymphoid cells within and around the ducts are typically larger than the rest. Other lymphoid cells are small, medium to large-sized cells with scant cytoplasm and atypical nuclei containing distinct nucleoli (Figure 1).

- In case no. 2, we notice a great variability of number and morphology of the lymphocytes: large lymphoid cells with pleomorphic nuclei, irregular coarse chromatin and prominent nucleoli, lymphoplasmacytic cells and multinucleated malignant lymphoid cells (Figures 2 and 3). Extensive necrotic areas (Figure 3) and inflammatory cells are present. Lymphoepithelial lesions, representing by the infiltration of ductal and epithelial structures by neoplastic B-cells are also seen (Figure 2).

Immunohistochemical findings

In case no. 1 by using the anticytokeratinic antibody we were allowed to reveal residual normal salivary ducts isolated within the tumor. In cytokeratin stain, the epithelial components are evident, the basement membrane is highlighted and the cytoplasm is positive (Figure 4). All the tumor cells presented an intense positive staining for LCA (Figure 5), and CD20 (Figure 6) on the cytoplasmic side of the cell membrane. The tissue samples were also evaluated as the immunostaining pattern for PCNA and p53, which revealed many tumoral cells immunopositive for PCNA (Figure 7) suggesting a history of abnormal mitotic processes; only few cells were considered positive for p53.

In the second case, the malignant lymphoid cells were immunoreactive for LCA also (Figure 8) and the B-cell immunophenotype was confirmed by immunopositivity for CD20 (Figure 9). The cytokeratin antibody (Figure 10) highlighted the remnants of lymphoepithelial lesion and the immunostain for PCNA and p53 (Figure 11) revealed large areas of intense-positive nuclei of malignant cells.

There was no evidence of positive immunoreaction for cytokeratins in tumor cells of both cases. A consistent cytokeratin staining was found in the normal salivary gland epithelium also allowing us to evaluate the lymphoepithelial lesions in the two cases (Figures 4 and 10). The tumor cells of the two cases taken into study were positive to the lymphoid markers LCA (Figures 5 and 8) and CD20 (Figures 6 and 9), with LCA was proved the lymphoid origin of the proliferative process and the immunopositivity to CD20 antibody showed the B-cell lineage of the cells. The p53 over expression tended to be higher in case no. 2.

The patient’s evolution was unfavorable in both
cases described here. On clinical follow-up, in case no. 1 the patient survived one year and two months after the surgery and the second patient (case no. 2) died eight months after the surgical treatment with liver metastasis. The particular nature of the two cases was the rarity of these tumors in our experience and the aggressive behavior showed. From a total of 204 salivary gland tumors studied (from 2002 to 2009), we diagnosed only two cases of primary malignant salivary gland lymphomas (Figure 12).

Figure 1 – Low-grade diffuse large B-cell mucosa associated lymphoid tissue (MALT) lymphoma with lymphoepithelial lesions (HE stain, ×400).

Figure 2 – EMZBCL with lymphoepithelial lesions. Note the pleomorphic lymphoid cells and prominent nucleoli (HE stain, ×400).

Figure 3 – EMZBCL. Atypical large lymphoid cells and necrosis (HE stain, ×400).

Figure 4 – Low-grade diffuse large B-cell MALT lymphoma. Positive staining for cytokeratin (MNF116) of normal salivary glands epithelial cells entrapped in malignant lymphoma areas (×400).

Figure 5 – Low-grade diffuse large B-cell MALT lymphoma. Immunostaining with LCA (2B11) (×200).

Figure 6 – Low-grade diffuse large B-cell MALT lymphoma. The diffuse lymphoid B-cell population is immunopositive for CD20 (L26) (×400).
Figure 7 – Low-grade diffuse large B-cell MALT lymphoma. Immunostaining with PCNA (PC-10) (×400).

Figure 8 – EMZBCL. Positive staining for LCA (2B11) proving the lymphoid origin (×400).

Figure 9 – The B-cell lineage of the EMZBCL lymphoma cells is established by intense stain for CD20 (L26). Note the salivary duct immunonegative for this antibody (×400).

Figure 10 – EMZBCL. Lymphoepithelial lesion highlighted by antibody to cytokeratin (MNF116) (×400).

Figure 11 – Intense positivity for p53 (DQ-7) of the high-grade EMZBCL lymphoma (×400).

Figure 12 – From a total of 204 cases of salivary glands tumors, primary malignant non-Hodgkin’s lymphomas represented 0.98%.

Discussion

Primary lymphomas of the salivary glands are very uncommon, only a small number of cases were described in the literature: Jaehne M et al. found one case of follicular lymphoma (FL) and eight cases of MALT-lymphoma [15], Kojima M et al. reported 12 cases of MALT-lymphoma, six of FL and two were diffuse large B-cell lymphoma (DLBCL) [14], Wolvius EB et al. examined four cases of FL and five cases of MALT-lymphoma [16], Dunn P et al. found
In all these studies the lymphomas originated mainly in the parotid and submandibular gland. Harris NL reported that 70% of lymphomas originate in the parotid and submandibular gland and <10% in minor salivary glands [17]. Kojima M et al. evaluated one case of MALT B-cell lymphoma of minor salivary gland of the oral cavity exhibiting tumor-forming amyloidosis [18], and Odell EW et al. reported two cases with low-grade MALT-lymphoma arising in primary Sjögren’s syndrome with hyalination and amyloidosis [19].

The diffuse lymphoid tissue can increase in some benign conditions and can resemble histologically non-Hodgkin’s lymphoma and because of that, a differential diagnosis between a benign condition and a malignant one must be made. Benign lymphoepithelial lesions (Mikulicz’ disease/lymphoepithelial sialadenitis/myoepithelial sialadenitis) appear with marked lymphoid infiltration and solid epithelial nests called epimyoepithelial “islands” which are infiltrated and surrounded by lymphoid cells of B-type [4, 17, 20].

This disease can remain localized in the salivary glands or be a part of Sjögren’s syndrome. Sjögren’s syndrome is a chronic inflammatory autoimmune disorder characterized by lymphocytic infiltration of lachrymal and salivary glands, which results in xerofthalmia, keratoconjunctivitis sicca and xerostoma; rheumatoid arthritis and higergammaglobulinemia are also found [4].

Sjögren’s syndrome appears in association with reactive and neoplastic lymphoproliferative diseases, frequently with B-cells. This association of benign lymphoepithelial lesions and Sjögren’s syndrome is believed to be a precursor lesion for extranodal marginal B-cell lymphoma of MALT-type [10].

An association between patients with MALT-lymphomas of salivary gland (+/- Sjögren’s syndrome) and scleroderma was also found [21]. Kutner’s tumor has sclerosing features simulating chronic sclerosing sialadenitis and the lymphocytes that infiltrate the epithelial component are mostly of B-type [22].

Kimura’s disease, which affects young adults belonging to Asian population, is a benign condition characterized by reactive lymphoid follicles, vascularization of germinal centers, eosinophilic infiltration, proliferating venules and important sclerosis [23].

Rosai-Dorfman’s disease presents sinus histiocytosis, massive lymph adenopathy and affects major salivary glands [24].

Another type of differential diagnosis can be made from a clinical point of view between MALT and follicular lymphomas – MALT lymphomas have a history of chronic inflammation as well as autoimmune disorders, but the follicular type is not associated with any chronic autoimmune disease and arises frequently from the submandibular gland [2, 8, 9, 25].

Diffuse large B-cell lymphoma (DLBCL) represents 15% of all non-Hodgkin’s lymphoma of the salivary glands [2].

Pan-B markers are expressed by the tumor. In our case, we use LCA (Figure 5) and CD20 (Figure 6) to demonstrate the lymphoid origin of the cells (LCA) and the B-cell lineage (CD20). It is however, known that MALT-lymphoma can progress to a diffuse large B-cell lymphoma but the criterion (on how) to define large-cell transformation is not universally accepted [1].

The cell population is mixed: large cells intermingled with small cells-and the right term in this situation might be MALT-lymphoma with increased large cell population (Figure 1). Recent studies have established that a large cell population bigger than 5% confers a slightly worse outcome for the tumor in question [26, 27], as seen in our lymphoma case, too.

Extranodal marginal zone B-cell lymphoma (EMZBCL) of salivary gland is a rare neoplasm-affecting adults with age raging between 55–65-year-old. Cases of EMZBCL at children and young adults have been reported. This lymphoma type is often associated with lymphoepithelial sialadenitis (LESA) in patients with Sjögren’s syndrome and it is postulated that low-grade EMZBCL develops because of an accumulation of mucosa-associated lymphoid tissue (MALT) that is acquired because of the autoimmune process. It occasionally occurs in patients without an autoimmune disease [2, 28]. The histopathological examination and the clinical data suggest that in our case no. 2 it is a high-grade EMZBCL-lymphoma (Figures 2 and 3) developed in the setting of lymphoepithelial sialadenitis without symptoms of Sjögren’s syndrome.

The prognosis of salivary gland lymphomas depends of histological type and clinical stage. It was observed that T-cell lymphomas and extranodal NK/T-cell lymphoma have a poor outcome. There are reports that diffuse large B-cell lymphoma with a nodal origin have a worse prognosis [6].

The prognosis of salivary gland EMZBCL-lymphoma is usually favorable especially if the tumors are localized at the time of presentation and demonstrate low-grade histology. For the high-grade EMZBCL of salivary gland the prognosis is still unclear, patients with EMZBCL-associated with LESA, which subsequently develop extensive extra salivary gland lymphoma or nodal large B-cell lymphoma, are known [2].

**Conclusions**

The primary malignant lymphomas of salivary gland cases represented only 0.98% of all salivary gland tumors diagnosed over a period of seven years. In the two cases that we evaluated we demonstrated the lymphoid origin of the cells using LCA and B-cell lineage with CD20.

The markers used to established the proliferation rate were PCNA and p53. Tumor cells with nuclear staining were considered positive. We evaluated nuclei from >400 tumor cells counted microscopically among the 5–8 random fields in each sample.
The expression of PCNA and p53 was considered intensely positive when ≥50% of cell population was positive. Case no. 1, the tumor cells were intensely positive (≥50%) for PCNA but few cells were positive for p53 (≤50%). Case no. 2 – the tumor cells stained highly positive at p53 and PCNA.

This highly positive appearance for p53 and PCNA suggests the abnormal mitotic processes and a high proliferative potential.

Case no. 1 was diagnosed with low-grade diffuse large B-cell mucosa associated lymphoid tissue lymphoma. In the second case, the clinical history, the histopathology and immunohistochemistry suggested a high-grade extranodal marginal zone B-cell lymphoma.

DLBCL and EMZBCL of salivary gland represent rare findings, only few cases have been reported in the literature.

The unique localization of these two cases requires a systematic work-up for a correct diagnosis and classification, which must integrate the histopathological and clinical findings with immunohistochemical evaluation.

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References


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