The Association of Warthin Tumor with Salivary Ductal Inclusions in Intra and Periparotid Lymph Nodes

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Abstract A predominant theory of the much debated histogenesis of Warthin tumor (WT) is that it arises from heterotopic salivary ductal inclusions (SDI) in parotid lymph nodes (LN). If this were the case, we might expect to see an increased number of SDI in the lymph nodes of patients with WT compared to controls. To test this, we compared the prevalence of SDI in patients with WT versus those with pleomorphic adenoma (PA). Cases of WT and PA were retrieved from the case files of the Department of Pathology at the QEII Health Science Centre, Halifax, NS, Canada. We then compared the prevalence of SDI in parotid LN between patients diagnosed with WT versus PA. 46 WT and 52 PA met our inclusion criteria. WT was significantly associated with an older age at

surgery (62.5 years vs 50.2 years, $p = 0.001$). 71.7 % of WT and 32.7 % of PA had inclusions in any LN. The presence of inclusion is a significant predictor for WT versus PA ($p = 0.019$). Where smoking status was available, 92.5 % of WT patients were smokers/ex-smokers, versus 55.1 % of PA ($p = 0.034$ for current smokers). Among PA, 44 % of smokers had inclusions compared with 22.7 % of non-smokers. SDIs are more frequent in parotid LN from patients with WT than PA. The high proportion of smokers among WT patients is consistent with prior studies. The results support the hypothesis that WT arises from SDIs. Individuals with more SDIs may be predisposed to WT.

Keywords Warthin tumor · Salivary ductal inclusions · Lymph nodes · Pleomorphic adenoma · Smoking

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Introduction

Warthin tumor (WT) is the second most common salivary gland neoplasm, typically occurring in the fifth to seventh decades of life. It is almost exclusively found in or near the parotid gland, rarely seen in minor salivary glands or lymph nodes in the neck [1, 2]. Smokers have an eightfold increased risk for developing the tumor. Traditionally it occurs more commonly in males, possibly reflecting the historically higher prevalence of smoking in men. Around 10 % of WT are multifocal and 10 % are bilateral. The treatment of WT is surgical resection, with a recurrence rate of about 2 % [3, 4].

The precise histogenesis of these tumors is not yet fully understood. The predominant theory for many decades is that the tumor arises from heterotopic salivary ductal inclusions in intraparotid and periparotid lymph nodes [5–8]. Others

Fig. 1 Photomicrograph of an intraparotid lymph node showing numerous salivary ductal inclusions (CK7 immunostain, 40X)

believe that WT is a reactive oncocytic epithelial proliferation arising from the parenchyma of the parotid, with a secondary lymphoid response [9].

We have observed that the presence of intra/periparotid lymph nodes with oncocytic ductal inclusions is common when examining parotid glands resected for WT in clinical practice. Occasionally, one encounters cases of WT with large numbers of inclusions in adjacent lymph nodes (Figs. 1, 2), which raises the question of whether patients with greater numbers of inclusions are predisposed to develop WT, or whether inclusions are acquired in conjunction with WT development. The effect of smoking is also unknown. In this study, we sought to determine the frequency of salivary ductal lymph node inclusions in a series of WT, in comparison with a series of control individuals with pleomorphic adenomas (PA), as well as to

Fig. 2 Higher power photomicrograph from the patient above showing salivary ductal inclusions with and without oncocytic metaplasia (H&E, 200X)

classify these inclusions into those with and without oncocytic metaplasia. Pleomorphic adenoma is the most common salivary gland tumor. Its pathogenesis is not thought to be related to salivary ductal inclusions, hence the presence of such inclusions should be similar or the same to those without a salivary gland tumor. The type and extent of surgery is the same for both WT and PA, as is the grossing protocol. Thus PA forms a large, relatively homogeneous cohort of cases for comparison.

Materials and Methods

Cases were chosen from the clinical case files of the QEII Health Sciences Centre in Halifax, Nova Scotia, a 1,000 bed tertiary care centre with a busy head and neck oncology service. We used the laboratory information system to search for diagnoses of Warthin tumor and pleomorphic adenoma between January 2001 and July 2008. Cases with co-existing malignant conditions such as metastatic squamous cell carcinoma were excluded, as were those in which the glass slides were unavailable for review. We collected epidemiological data including patient's sex, age at the time of operation and their smoking status, when available.

In order to standardize the analysis, we used the following criteria: (1) a lymph node was defined as a collection of lymphoid tissue of any size with a discrete capsule and a subcapsular sinus; (2) inclusions were defined as collections of luminal and abluminal columnar-cuboidal ductal cells that were completely enclosed within a node by lymphoid tissue; acinar cells and ducts admixed with fat at the hilum of a node were not included; (3) as in most cases it was not possible to determine the exact number of nodes in a specimen, the number of pieces of nodal tissue per case was recorded as a proxy measure; (4) WT was defined as an aggregate of lymphoid tissue >1 cm in greatest dimension containing proliferating oncocytic epithelium; (5) lymph node tissue close to a WT was considered distinct from the tumor if it was separated from the tumor by normal parotid tissue, skeletal muscle or large vessels, not just by fat.

For each case, the number of lymph node sections and their location was recorded, including whether it was intra/periparotid or cervical neck nodes. Salivary ductal inclusions were subdivided into those with or without oncocytic change, or with both. The surrounding salivary gland parenchyma was also examined and the presence of oncocytic metaplasia or ductal hyperplasia was recorded. Statistical comparisons were performed using binary logistic regression to evaluate the independent effects of each variable. Analyses were performed using SPSS for windows v. 20.

Results

There were 74 cases with a diagnosis of WT between January 2001 and July 2008. Seventy-seven consecutive PA were chosen from the latter portion of the study period to serve as controls. Forty-six cases of WT and fifty-two cases of PA were included by the above criteria. Among the excluded WT cases, 5 had co-existing malignancies, 3 had co-existing pleomorphic adenoma, 1 had no glass slides in the file, 1 was a biopsy only and 18 had no nodal sections included in the slides. Two patients with WT had a recurrence within the study period and these were considered as distinct cases. Among the excluded PA cases, 2 had co-existing malignancies, 3 had a co-existing WT (same cases as above), 1 was a biopsy only, 1 had no glass slides in the file, and 18 had no nodal sections included in the slides.

In the WT group, the average patient age at surgery was 62.5 years (range 37.8–85.2), compared with 50.2 years (range 16.4–82.3) in the PA group ($p = 0.001$) (Table 1). The WT group had a male to female ratio of 1:1.3 and the PA group had a ratio of 1:1.6. Smoking status was available for 40 patients with WT and 49 with PA. 92.5 % (37 of 40) of patients with WT were smokers or ex-smokers, compared with 55.1 % (27 of 49) of patients with PA. There is a significant association in WT with current smokers compared to those in PA ($p = 0.034$).

A total of 383 and 270 sections of lymph node tissue were counted for WT and PA, respectively. WT cases had an average of 6.13 nodal sections per case compared with 3.50 sections per case for PA. Of all nodal sections identified, 85 of 383 (22 %) and 30 of 270 (11 %) were found to have inclusions for the WT and PA groups, respectively. 71.7 % (33/46) of WT patients and 32.7 % (17/52) of PA patients had salivary ductal inclusions in any lymph node tissue. The presence of inclusions is a significant predictor of WT versus PA (odds ratio 3.871, $p = 0.019$) in the multivariate model.

Among WT subjects with inclusions, 21 of 33 (63.6 %) had oncocytic inclusions (exclusively or in combination with non-oncocytic inclusions) compared with 3 of 17

(17.6 %) in the PA group. Thus overall 21 of 46 (45.7 %) WT subjects with nodal tissue had oncocytic inclusions, compared with just 3 of 52 (5.8 %) PA subjects. Oncocytic inclusions were significantly associated with smoking in a multivariate model.

The vast majority (92.5 %) of WT patients were smokers/ex-smokers, therefore the relationship between smoking and the presence or absence of inclusions could not be determined. In the PA group, 12 of 27 smokers (44 %) had ductal inclusions, compared with only 5 of 22 non-smokers (22.7 %). However, this difference was not statistically significant. 2 of 3 PA patients with oncocytic inclusions were ex-smokers, one was a non-smoker.

For the PA patients, we calculated the average number of lymph node sections per case for the smokers/ex-smokers (4.9 per case, $N = 27$) and non-smokers (6.1 per case, $N = 22$). The difference was not statistically significant. As there were so few non-smokers among the WT patients, this was not considered to be a relevant calculation. Foci of oncocytic metaplasia were noted in the surrounding parotid tissue in 4 cases each of WT and PA (NS).

Discussion

The pathogenesis of WT has been debated for decades, with the predominant hypothesis being that these tumors arise from a proliferation of oncocytic epithelium and lymphoid tissue derived from entrapped salivary ductal tissue within parotid lymph nodes [5–8]. Despite this, the prevalence of salivary ductal inclusions in lymph nodes of patients with parotid WT versus controls has not previously been studied.

The results of our study support this hypothesis. We found that salivary ductal inclusions were significantly more common in the intraparotid and periparotid lymph nodes of patients with Warthin tumor than in those with pleomorphic adenoma. Oncocytic inclusions in particular, were very uncommon in PA patients (5.8 %), whereas they occurred in nearly half of WT patients in whom parotid lymph node tissue was identified.

As expected, WT among our population is a predominantly disease of smokers, with a history of smoking in 92.5 % of the WT patients in this study. Interestingly, both WT and PA were more common in women than men. For WT, this could reflect a balancing of smoking rates between women and men in recent years.

The exact role of salivary ductal inclusions in the pathogenesis of WT is unknown. Patients with more inclusions may be more prone to develop WT. The occasional case of WT in a patient with numerous non-oncocytic inclusions, such as the one illustrated in our figures, suggests that this may be the case. It is not possible to entirely exclude the possibility that inclusions are acquired, proliferate and/or

Table 1 Binary logistic regression showing associations of the variables with Warthin tumor

Variable	Odds ratio	95 % CI	<i>P</i> value
Age	0.908	0.865–0.953	0.001
Current smoker	0.034	0.239–0.897	0.034
Ex-smoker	3.761	0.832–17.006	0.835
Never smoked	0.174	0.015–2.037	0.163
Presence of inclusions	3.871	1.253–11.966	0.019
Sex	1.990	0.620–6.386	0.248
Tumor size	1.011	0.755–1.355	0.941

Sex: reference set to female

Bold values indicate statistically significant

undergo oncocyctic metaplasia as an aging phenomenon, thereby explaining an increased frequency in the older WT group. However, we favor that smoking, not age, stimulates ductal hyperplasia and oncocyctic metaplasia in pre-existing intraparotid lymph nodes inclusions. With respect to whether inclusions are congenital or acquired, we found that inclusions were more common among smokers than non-smokers among our PA patients, but the difference did not reach statistical significance. Therefore we cannot comment on this based on our data.

The parotid is not the only upper aerodigestive tract organ that can exhibit Warthin-like changes in smokers. Oncocyctic metaplasia, cyst formation and papillary hyperplasia are also seen in oncocyctic papillary cystadenomas of the larynx, a condition which is more common among smokers [10]. Essentially, these lesions can be regarded as a laryngeal counterpart to WT.

We found that on average, there were more sections of parotid lymph node tissue in the WT cases. Intraparotid lymph nodes were not dissected out separately, so the same node may have been sampled more than once. As the same grossing protocol was used for all tumors, there should have been no bias towards greater lymph node sampling in the WT cases. It is tempting to speculate that smoking also stimulates lymphoid hyperplasia in parotid lymph node tissue, similar to the situation in the nasopharynx (so-called adult onset adenoidal hyperplasia) [11]. If this were the case, the higher percentage of smokers in the WT group might lead to a greater volume lymphoid tissue with more being sampled. We considered that the greater number of node sections in the WT cases might lead to an increased pick-up rate of inclusions for a given patient. However overall, inclusions were still twice as common in node sections of the WT patients.

Taking the above findings into account, it is possible that a combination of both epithelial and lymphoid hyperplasia can best explain the pathogenesis of WT. A similar explanation has been proposed for the development HIV-related parotid cysts, in which the florid lymphoid hyperplasia seen in HIV-related lymphadenopathy is thought to promote cyst formation from intra nodal ductal inclusions [12].

Clonality studies on WT have mostly suggested that it is of non-neoplastic in nature [13, 14], although this is a complex and controversial topic. Under exogenous stimulations such as smoking, transition from hyperplasia and metaplasia to neoplasia may occur [14], as is thought in some other organs (for example in thyroid multinodular goiter) [15, 16].

In summary, our results indicate that salivary ductal inclusions and oncocyctic metaplasia are more common in patients with WT than in those with PA. We favor that a combination of lymphoid hyperplasia and ductal

hyperplasia and metaplasia, stimulated by smoking and acting on pre-existing inclusions, drives the formation of WT. Neoplastic transformation may occur subsequent to these alterations.

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Conflict of Interest None.

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