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CLINICAL RECORD

Case report of a p16^{INK4A}-positive branchial cleft cyst

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Abstract

Objective: To report the occurrence of a concurrent oropharyngeal papilloma and branchial cleft cyst linked by p16^{INK4A} and human papillomavirus immunohistochemistry.

Case report: A 42-year-old woman presented with a 1-month history of a left lateral neck mass. Contrast enhanced computed tomography showed a hypodense lesion 20 mm in diameter anteromedial to the left sternocleidomastoid muscle. Ultrasound-guided fine needle aspiration suggested a branchial cleft cyst. Panendoscopy was performed at the time of neck mass removal, and a papillomatous lesion was removed from the left hypopharynx. Histopathological analysis showed the neck lesion to be a branchial cyst containing lymphoid tissue, and the oral lesion to be a squamous papilloma. Immunohistochemical analysis showed both the branchial cleft cyst and papilloma to be positive for p16^{INK4A} expression and human papillomavirus DNA.

Conclusion: Histological and immunohistochemical analyses support the cystic transformation of lymph nodes, or the 'Inclusion Theory', as the aetiology of branchial apparatus anomalies, and raise the possibility that human papillomavirus infection may play a much larger role in disease of the head and neck than previously supposed.

Key words: Papillomaviridae; p16^{INK4A}; Branchioma

Introduction

Considerable controversy remains regarding the aetiology of the lateral cleft cyst, sinus or fistula since it was first described in 1785;[1] however, it is widely believed to be branchiogenic in origin.[2] Some authors have rejected the branchiogenic theory in favour of the growing evidence for a cystic lymph node origin.[1,3,4] Lateral cervical cysts are reported to be aetiologically distinct from sinuses and fistulae, and many mechanisms are proposed to be involved in their pathogenesis.[1,2,5] There is little detailed published information about the incidence or prevalence of branchial anomalies; however, it is an uncommon condition, which partly explains the ongoing controversy surrounding its aetiology.

Papillomaviridae, or the human papillomaviruses (HPVs), are now a well-recognised cause of oropharyngeal cancers.[6] In a recent summary by the National Cancer Institute State of the Science, Adelstein *et al*. discussed the continuing evolution of our understanding of the significance of HPV infection in the setting of head and neck cancer.[7] They went on to suggest that HPV may serve as a future therapeutic target.[7] Human papillomavirus involvement in benign head and neck papillomas is also well known. Thus, it is reasonable to consider whether HPV infection may play a role in other head and neck diseases.

Case report

A 42-year-old woman presented with a 1-month history of an enlarging left lateral neck mass. She also suffered from recurrent bronchitis, gastric reflux, dyslipidaemia and type 2 diabetes mellitus, which was treated with oral hypoglycaemic drugs. Examination identified a palpable left lateral neck mass approximately 3 cm in diameter. A computed tomography scan with contrast showed a hypodense lesion of dimensions 20 mm × 24 mm × 30 mm at the division of the internal and external carotid arteries anteromedial to the left sternocleidomastoid muscle (Figures 1 and 2). Ultrasonography showed this to have an avascular cystic structure. Under ultrasound guidance, 10 ml of purulent fluid was aspirated. Cytological analysis revealed abundant neutrophil polymorphs, squamous cells, anucleate squames, histiocytes and proteinaceous material, consistent with an inflamed branchial cyst. No malignant cells were seen. An aspirate of the lesion was positive for p16^{INK4A} but negative for HPV.

The patient underwent panendoscopy, during which a papillomatous lesion was noted in the left hypopharynx. The pharyngeal lesion was biopsied, and then the cystic neck mass was removed via en bloc left level two neck dissection. No cutaneous tracts or deep extensions from the neck mass were noted during the operation. The biopsy specimen was sent for histopathological, p16^{INK4A} and HPV analysis. The patient's recovery was uneventful.

The pharyngeal lesion was diagnosed as a squamous cell papilloma with typical histopathology. Histological analysis of neck mass sections showed a benign cyst lying either within or adjacent to a lymph node, predominantly lined

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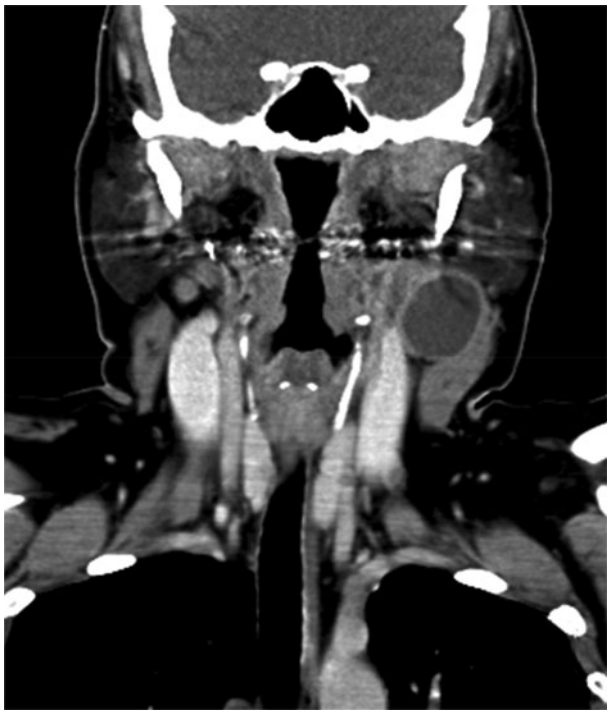

FIG. 1
Coronal computed tomography scan showing a hypodense lesion in the left neck.

by bland stratified squamous cell epithelium and containing keratin-like material and sparse inflammatory cells, consistent with a benign branchial cyst. The surrounding connective tissue contained nine lymph nodes, all of which showed benign reactive changes. Both the squamous cell papilloma and the branchial cyst were positive for $p16^{INK4A}$ expression and HPV infection. Immunohistochemical analysis showed occasional $p16^{INK4A}$-positive cells within the squamous cell papilloma epithelium and the branchial cyst wall.

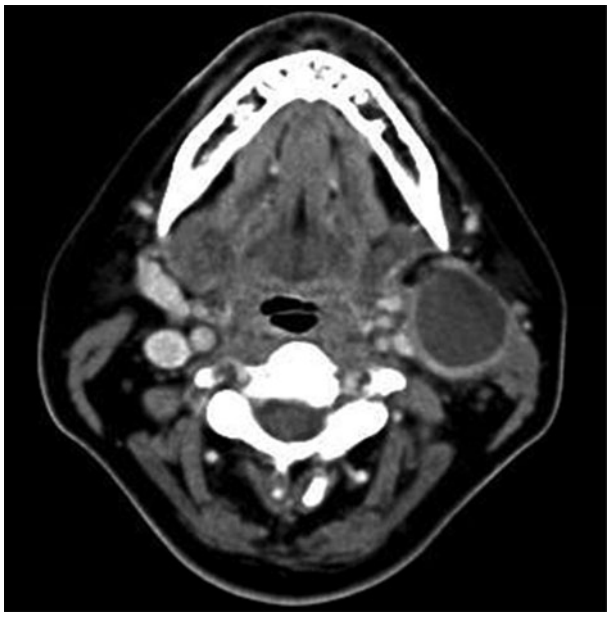

FIG. 2
Axial computed tomography scan showing a hypodense lesion in the left neck.

Ethical approval for publishing the case report was obtained from the Austin Health Office for Research on behalf of the Austin Health Human Research Ethics Committee.

Discussion

At least five theories exist regarding the aetiology of lateral cervical cysts, sinuses and fistulae: these are thought to derive from the remnants of embryological structures or the cystic transformation of lymph nodes. However, it is possible that multiple mechanisms may be responsible.[1]

The 'Branchial Theory', described by Ascherson in 1832, holds that branchial apparatus anomalies result from entrapped remnants of branchial cleft components which have failed to obliterate during normal development. He proposed the 'Pre-cervical Sinus Theory', which claimed that branchial apparatus anomalies arise from trapped cervical sinus remnants retained during embryogenesis. The 'Vestigial Remnant Theory', an expansion of the first two theories, proposes that any part of the branchial apparatus (such as the cleft or pouch) or the cervical sinus can give rise to a lateral cervical cyst, sinus or fistula if it does not obliterate. Alternative theories suggest that a persistent thymopharyngeal duct could lead to cyst formation.[1]

The 'Inclusion Theory' states that lateral cervical cysts, sinuses and fistulae are derived from the cystic transformation of lymph nodes induced by epithelium entrapped within them.[1] Thus, branchial or parotid gland epithelium, which develops at the same time as the lymph nodes, is entrapped or translocated postnatally from the palatine tonsils.[1,3] However, there are a number of problems with this theory: lymph nodes are recognised inclusions within the parotid tissue but few branchial cysts are located in this area; salivary gland inclusions are rare in the areas that branchial cysts are common[9]; and cystic transformation of lymph nodes is not recognised elsewhere in the body.[10] Most of the support for the 'Inclusion Theory' comes from histopathological studies.[1] In a review of branchial anomaly aetiology, Maran and Buchanan support the 'Inclusion Theory' because most branchial cysts, sinuses or fistulae contain lymphoid epithelium in their walls, they are found in structures not derived from the branchial apparatus, and embryological evidence is sparse for the branchial theory.[10] Histopathological analysis in this case supports the 'Inclusion Theory' as the aetiology for branchial anomalies because it shows a cystic mass lined by stratified squamous epithelium containing lymphoid tissue.

In the current case, immunohistochemical staining showed $p16^{INK4A}$ expression in the papilloma epithelium and the wall of the cystic neck lesion. Similar $p16^{INK4A}$ staining patterns have been identified in branchial cleft cysts.[11] Epithelial $p16^{INK4A}$ expression can result from HPV infection, and is used as a surrogate marker of this infection. Human papillomavirus E7 gene products cause functional inactivation of the retinoblastoma protein, which is a negative regulator of $p16^{INK4A}$, resulting in $p16^{INK4A}$ over-expression.[7]

The 'Inclusion Theory' holds the branchial cyst to be the cystic transformation of a lymph node. If this is correct, then it is plausible that oral mucosa infected with HPV could infect local lymphatic drainage structures. In the present case, the branchial cyst was found in the draining lymphatic basin of the papilloma. There seems to be a clear link between $p16^{INK4A}$ over-expression and HPV positivity in the papilloma and in the branchial cyst, which supports the 'Inclusion Theory'.

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If branchial cysts were truly derived from branchial apparatus, then to be infected with $p16^{INK4A}$ they would have to directly communicate with, or drain, HPV-infected epithelium. This case presents a branchial cyst with no apparent communication with a mucosal surface. Thus, this case does not support an embryological origin for the branchial cyst. It is worth noting that the most commonly infected site in the pharynx or oral cavity (as indicated by $p16^{INK4A}$ positivity) is the tonsils, and that type II branchial fistulae and sinuses are traditionally considered to communicate with the tonsil. Thus, communication between a subclinical sinus or fistula tract and HPV-infected epithelium cannot be ruled out. However, in this case, the papilloma was situated well posterior to the tonsil, in an area not traditionally considered to be of branchial apparatus origin.

Human papillomavirus and $p16^{INK4A}$ testing is complex and in Adelstein *et al*., Gravitt stresses that the test results are greatly influenced by virus type, sample quality and detection method.[7] Pai *et al*. found 6 out of 21 branchial cleft specimens to be positive for $p16^{INK4A}$ expression; polymerase chain reaction identified HPV DNA in 7 out of 19 specimens. However, testing by in situ hybridisation showed that HPV was absent in all samples.[7] In Adelstein *et al*., Westra points out that HPV positivity (as determined by the polymerase chain reaction) does not usually show whether there is a biologically meaningful HPV infection; instead, it detects DNA from numerous strains of HPV. There is a discrepancy of 25 per cent between $p16^{INK4A}$ analysis and in situ hybridisation (for HPV16) when large numbers of head and neck squamous cell carcinomas are tested: often, $p16^{INK4A}$ expression is positive and HPV in situ hybridisation results are negative owing to non-HPV 16 strains causing $p16^{INK4A}$ over-expression and to $p16^{INK4A}$ expression being an imperfect surrogate marker.[7] Interestingly, $p16^{INK4A}$ can be detected in normal tissues, and its expression increases with age because of its role in cellular senescence.[12] The discrepancies between $p16^{INK4A}$ and HPV testing of the FNA and tissue samples are most likely accounted for by sampling and testing factors. These data highlight some of the current limitations of $p16^{INK4A}$ and HPV testing, as well as the need to improve the accuracy of HPV testing and its application.

- **Branchial apparatus anomalies may arise from epithelium-induced cystic lymph node transformation ('Inclusion Theory') or from branchial apparatus remnants**
- **Concurrent oropharyngeal squamous cell papilloma and branchial cleft cyst were positive for $p16^{INK4A}$ and Human papillomavirus (HPV) DNA**
- **This case supports the 'Inclusion Theory'**
- **HPV infection may play a much larger role in head and neck disease than previously thought**
- **The standard of care for branchial cyst management should include panendoscopy**

The oropharyngeal papilloma was noted by panendoscopy at the time of operation and not at pre-operative nasendoscopy. This raises the question of whether the standard of care for the management of branchial cysts should include panendoscopy. Panendoscopy might help further answer the question of aetiology; however, its clinical importance may be minimal because it is unlikely to alter the management strategy. The immunohistochemical data in the present case, indicating an oropharyngeal papilloma and branchial cyst, provide elegant but circumstantial support for the 'Inclusion Theory' of branchial anomaly aetiology. This case also raises the possibility that HPV infection may play a much larger role in head and neck disease than previously known; however, there are clearly limitations to HPV testing.

References

1 Golledge J, Ellis H. The aetiology of lateral cervical (branchial) cysts: past and present theories. *J Laryngol Otol* 1994;**108**:653–9
2 Glosser JW, Pires CA, Feinberg SE. Branchial cleft or cervical lymphoepithelial cysts: etiology and management. *J Am Dent Assoc* 2003;**134**:81–6
3 Bhaskar SN, Bernier JL. Histogenesis of branchial cysts; a report of 468 cases. *Am J Pathol* 1959;**35**:407–43
4 Chandler JR, Mitchell B. Branchial cleft cysts, sinuses, and fistulas. *Otolaryngol Clin North Am* 1981;**14**:175–86
5 Shinkwin C, Whitfield BC, Robson AK. Branchial cysts: congenital or acquired? *Ann R Coll Surg Engl* 1991;**73**:379–80
6 D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM *et al*. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;**356**:1944–56
7 Adelstein DJ, Ridge JA, Gillison ML, Chaturvedi AK, D'Souza G, Gravitt PE *et al*. Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008 Washington, D.C. *Head Neck* 2009;**31**:1393–422
8 Benson MT, Dalen K, Mancuso AA, Kerr HH, Cacciarelli AA, Mafee MF. Congenital anomalies of the branchial apparatus: embryology and pathologic anatomy. *Radiographics* 1992;**12**:943–60
9 Little JW, Rickles NH. The histogenesis of the branchial cyst. *Am J Pathol* 1967;**50**:533–47
10 Maran AG, Buchanan DR. Branchial cysts, sinuses and fistulae. *Clin Otolaryngol Allied Sci* 1978;**3**:77–92
11 Pai RK, Erickson J, Pourmand N, Kong CS. p16(INK4A) immunohistochemical staining may be helpful in distinguishing branchial cleft cysts from cystic squamous cell carcinomas originating in the oropharynx. *Cancer* 2009;**117**:108–19
12 Nielsen GP, Stemmer-Rachamimov AO, Shaw J, Roy JE, Koh J, Louis DN. Immunohistochemical survey of p16INK4A expression in normal human adult and infant tissues. *Lab Invest* 1999;**79**:1137–43

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