Head and neck squamous cell carcinoma in pregnant women

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ABSTRACT: *Background.* The aim of this study was to investigate oral cancer in pregnant women, a rare but therapeutically challenging patient subset.

Methods. After institutional review board approval, an EMERSE search was used to identify all women treated at the University of Michigan from 1998 to 2010 with head and neck squamous cell carcinoma (HNSCC) during pregnancy. This identified 4 patients with tongue cancer. Biomarkers and human papillomavirus (HPV) were assessed by immunohistochemistry and multiplex PCR/mass spectrometry, respectively.

Results. Two patients responded well to therapy and are alive more than 10 years after diagnosis; 2 patients died of disease. All tumors overexpressed EGFR and Bcl-xL, 3 of 4 overexpressed c-Met, both tumors that progressed overexpressed p53. All tumors were negative for HPV, p16, estrogen receptor, progesterone receptor, and HER-2.

Conclusions. Biomarkers of aggressive tumors (high EGFR, c-Met; high Bcl-xL-low p53) did not correlate with outcome. Additional studies are needed to determine whether perineural invasion, delay in diagnosis, and p53 overexpression are factors in poor survival. © 2012 Wiley Periodicals, Inc. *Head Neck* **35: 335–342, 2013**

KEY WORDS: oral cancer, biomarkers, HPV, pregnancy, risk factors

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is typically considered to be a disease that predominantly affects older men, with a male:female ratio of approximately 4:1.[1] Long-term alcohol and tobacco use have been identified as the traditional risk factors for this disease. Interestingly, recent trends have shown an increase in the incidence of HNSCC in younger patients without these risk factors, and there is controversy over whether these represent a more aggressive form of cancer.[2] Recently, an increased incidence of HNSCC of the tongue in female patients has been described, which is partially responsible for a noted fall in the male:female ratio of patients affected by this disease.[3] Therefore, although it is still rare for a young woman of reproductive age to be diagnosed with HNSCC, recent developments suggest an increasing risk for this population.

When HNSCC is diagnosed in a pregnant patient, clinicians and patients are faced with the challenge of balancing maternal and fetal health. Although early detection and intervention are key, it is also important to weigh the risks of diagnostic and treatment modalities to the fetus. The patient is faced with difficult ethical decisions, and the clinician is often tasked with providing both optimal treatment for the cancer and protection of the fetus.

There is a paucity of data regarding the etiology of cancers of the head and neck in pregnant women. The studies that do exist are primarily case reports discussing the challenges that clinicians face in administering treatment that is of maximal benefit to the patient and minimal risk to the fetus.[4–7] Although the hypothesis that these tumors are hormonally induced during pregnancy seems logical, it has not yet been determined whether a biological predisposition to HNSCC during pregnancy exists. It seems plausible that these patients may have from a latent human papillomavirus (HPV) that becomes active and oncogenic during pregnancy. However, none of the cases

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reported in the literature has been assessed for HPV status. We report a series of cases in which pregnant women presented with HNSCC to the University of Michigan Department of Otolaryngology–Head and Neck Surgery between 1998 and 2010. These patients' tumors were analyzed for biomarker expression and HPV status.

MATERIALS AND METHODS

Patient population

Permission from the institutional review board (IRB) for human studies was granted to identify patients that presented to the Department of Otolaryngology between 1998 and 2010 with head and neck squamous cell carcinoma during pregnancy using the University of Michigan's Electronic Medical Record Search Engine (EMERSE). This query identified 4 patients. IRB approval was also granted for use of existing patient specimens and data.

DNA isolation

DNA were extracted from a core of formalin-fixed, paraffin-embedded tissue taken from each pretreatment biopsy or surgical resection specimen for HPV analysis. The core of tissue was deparaffinized, then DNA was isolated in accord with the manufacturer's protocol (QIAmp DNA Mini Kit; Qiagen).

Immunostaining

Slides containing tissue sections from the resected tumors were deparaffinized, rehydrated, and peroxidase-quenched (Dako Cytomation, Glostrup, Denmark) as described previously.[8] All slides were incubated in Antigen Retrieval Solution (Dako Cytomation) for 40 minutes in 92°C water bath with a buffer change midway and allowed to cool to room temperature for 20 minutes. For epidermal growth factor receptor (EGFR), an additional antigen retrieval step was performed with pepsin incubation for 10 minutes at 37°C. Horse serum was used for blocking (30 minutes at room temperature). Primary antibodies, prediluted EGFR/31G7, and 1:300 dilution of c-Met/3D4 (Zymed Laboratories, South San Francisco, CA), 1:100 dilution of p53/DO1 and 1:100 dilution of Bcl-xL/7D9 (Lab-Vision, Fremont, CA), prediluted p16/E6H4 (CINtec Histology, Westborough, MA), 1:200 dilution of ERa/SP1 (BioCare Medical, Concord, CA), 1:200 dilution of ERb/88 (Biogenex, San Ramon, CA), 1:50 dilution of PR/636 and 1:100 dilution of cErbB2 (HER2) (Dako, Carpinteria, CA), were allowed to incubate overnight at 4°C. Slides were washed and secondary antibodies linked to avidin/biotin peroxidase (ABC Kit; Vector Laboratories, Burlingame, CA) were used to detect primary antibody binding. All stained slides were reviewed and scored by an experienced pathologist using an intensity scale from 1 to 4 (1 = no staining; 2 = weak; 3 = moderately strong; 4 = intense signal) and a 4-point proportion-positive scale, where 1 $\leq$10%; 2 = 11% to 25%; 3 = 26% to 50%; and 4 = 51% to 100%. The intensity and proportion were multiplied together to give an overall immunohistochemistry (IHC) score from 0 to 16.

HPV analysis

HPV analysis from the extracted DNA from pretreatment biopsies or surgical specimens was accomplished using a sensitive and quantitative real-time polymerase chain reaction (PCR) with primers that are specific to the HPV-E6 region of each of the 15 most common high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73). HPV analysis was carried out using the Attosense multiplex PCR–mass spectrometry method developed at the University of Michigan and licensed to Sequenom as previously described.[9]

RESULTS

Case reports

Patient 1. A 26-year-old white woman with no significant past medical history presented to our clinic with pain on the right side of her tongue, mild dysphagia, and left-sided otalgia. She had recently found out she was pregnant. The patient had no history of alcohol use but had a 6-pack-year tobacco use history. She was no longer smoking at the time of presentation. She had no family history of cancer. Her exam was notable for a large, ulcerated, tender lesion of the right lateral tongue with palpable induration extending across the midline. Biopsy of her right lateral tongue revealed well-differentiated invasive squamous cell carcinoma (SCC). Preoperative CT scan revealed an irregular 3.5-cm $\times$ 2.5-cm mass in the right posterior tongue that extended inferiorly. Multiple scattered, enlarged lymph nodes were noted bilaterally.

Following elective termination of her pregnancy, the patient underwent bilateral selective neck dissections of levels I through IV, subtotal glossectomy, and an anterolateral thigh fasciocutaneous free flap transfer. Tumor pathology revealed a 5-cm well-differentiated, ulcerative SCC with a depth of invasion of 2 cm. There was extensive perineural invasion and several unilateral lymph nodes positive for malignancy, some of which had extracapsular extension. The final tumor margins were negative, and the tumor was classified as T4aN2bM0 (Figure 1A), with a type 3 pattern of invasion.[10]

The patient's postoperative recovery was complicated by an oral–cutaneous fistula. Adjuvant platinum/paclitaxel chemotherapy and radiation to a dose of 60 Gray (Gy) were recommended. After receiving 3 doses of chemotherapy and 5.4 Gy of radiation, she was unable to continue making her scheduled appointments.

Approximately 6 months after her surgery, the patient presented to the emergency department with neck swelling and was admitted for a large neck abscess. Biopsy revealed recurrent invasive well- to moderately-differentiated SCC. Position-emmision tomography (PET) scan demonstrated FDG-avidity in the right neck but was negative for distant lesions. Her recurrence was deemed unresectable, and she was started on a palliative chemoradiation regimen. The patient subsequently developed lung metastases and passed away approximately 1 year after diagnosis.

Patient 2. A 33-year-old white woman presented with soreness of the left tongue and a 1-month history of a neck mass. She first noted these symptoms during her last month of pregnancy. After delivery, she continued to

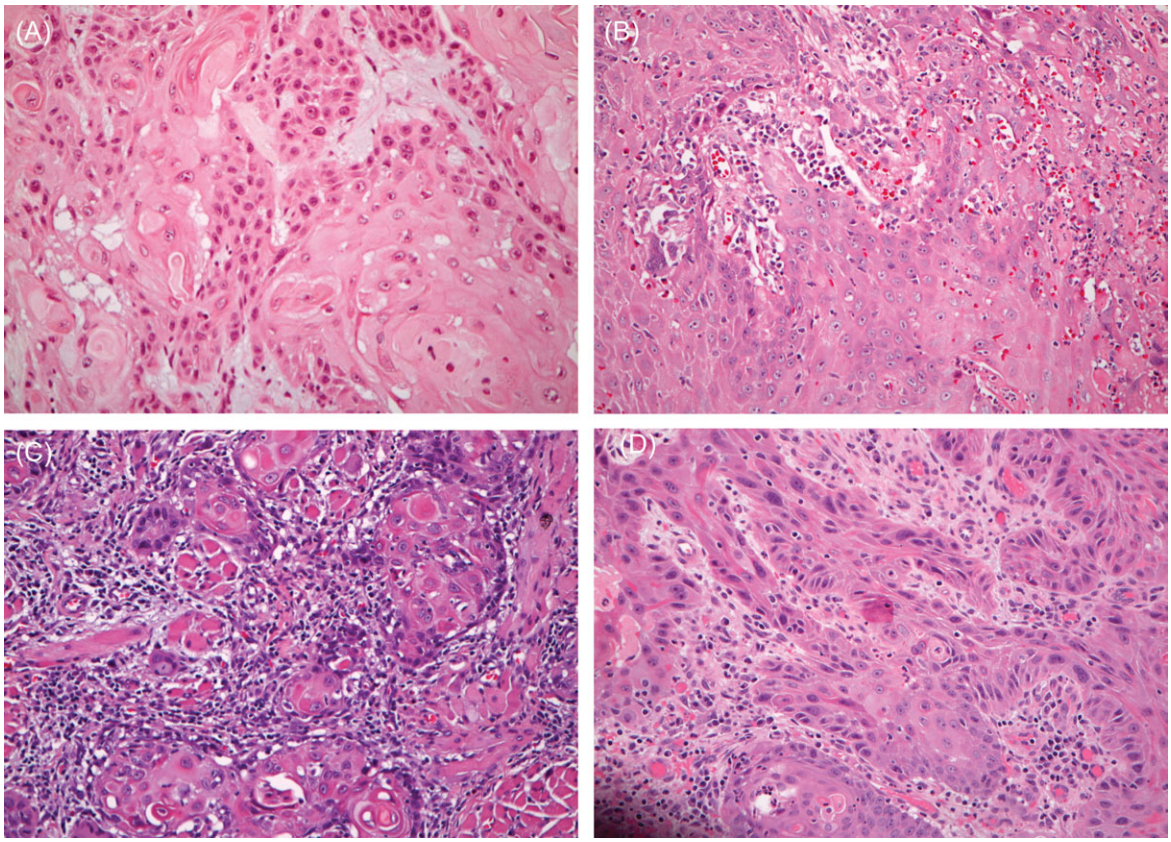

FIGURE 1. Hematoxylin and eosin staining of the invasive squamous cell carcinoma tissue from each of the cases. Case 1 (A), case 2 (B), case 3 (C), case 4 (D). (A–D, original magnification ×200). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

notice increasing soreness and fullness on the left side of her tongue as well as fullness in the left upper neck. Notably, she had no history of tobacco or alcohol use.

Physical exam revealed a 1.7-cm × 3.0 cm area of ulceration and erythroplakia on the left lateral edge, surrounded by a 0.5 cm rim of submucosal firmness. There was no involvement of her floor of mouth or base of tongue. Her left neck revealed a 4.0-cm × 3.0-cm mass in the submandibular region, levels I and II. Biopsy confirmed SCC of the tongue.

She underwent a partial glossectomy and left selective lymph node dissection. The primary tumor was a moderately differentiated, 2.3 cm mass with pushing borders. There was no vascular invasion. She had several positive level II lymph nodes, some with extracapsular spread. Her tumor was classified as T2N2bM0 (Figure 1B) with a type 3 pattern of invasion.[10] She received postoperative radical external beam radiation therapy. She has returned for follow up every 6 to 12 months and was free of disease at her most recent appointment 12 years after diagnosis.

Patient 3. A 30-year-old white woman presented with a history of intermittent soreness on her left lateral tongue, which over several months had worsened until her pain became persistent and interfered with her ability to eat. She had recently found out she was pregnant. She had no other specific complaints related to the head and neck. She had no history of tobacco or alcohol use, and no family history of cancer. Her exam revealed a 2 cm mucosal lesion on the lateral border of her left tongue. No apparent mass or induration was present upon palpation of the tongue and no other masses were noted. A biopsy confirmed SCC of her left lateral tongue with a depth of invasion of 2 mm. MRI showed a 3 to 3.5 cm lesion of the tongue with underlying skeletal muscle invasion without evidence of cervical adenopathy.

She underwent a left hemiglossectomy, a left selective neck dissection of levels I–III, and a tracheostomy. Pathology revealed a 0.7-cm moderately-differentiated SCC with infiltrative borders and a depth of invasion of 0.9 cm. Extensive perineural invasion was present. All cervical lymph nodes were negative. There were positive margins, requiring reoperation, after which clear margins were obtained. The tumor was classified as T2N0M0 (Figure 1C) with a type 4 pattern of invasion.[10] Postoperative radiation was considered but not performed.

Approximately 4 months later, the patient noted intermittent pain that became progressively worse. Imaging was postponed until after delivery. At that time, a PET scan showed FDG-avidity in the posterolateral tongue extending into the base of tongue and floor of mouth. Options for therapy included a total glossectomy and possible laryngectomy versus radiation therapy with chemotherapy. After discussion the latter treatment option was chosen.

She began a platinum/paclitaxel chemotherapy regimen followed by 70 Gy of radiation. Three months after

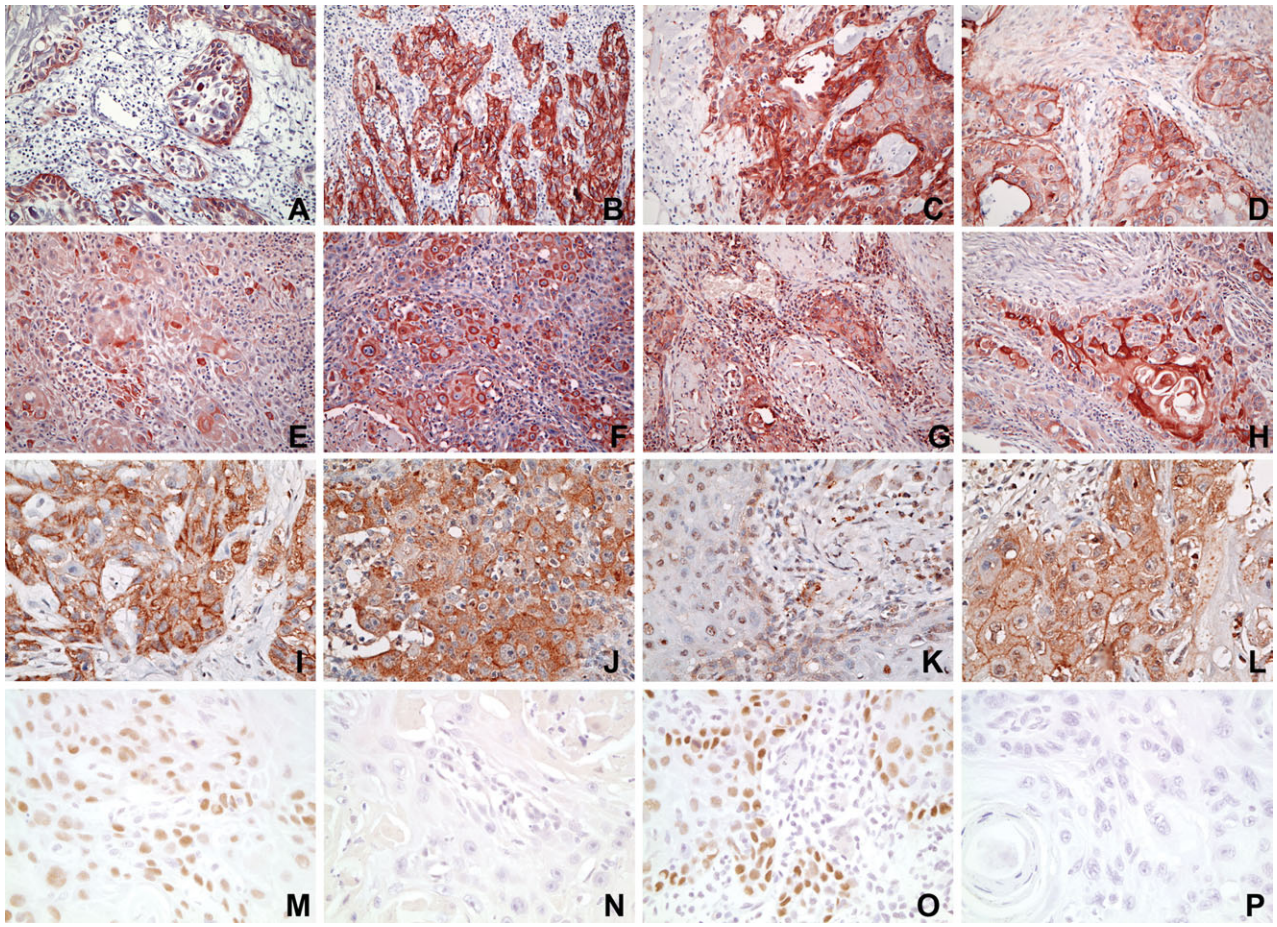

FIGURE 2. Staining for EGFR (A–D), BcL-xL (E–H), c-Met (I-L), and p53 (M–P) of the squamous cell carcinoma primary tumor from each patient. (A–H, original magnification, ×200; I–P, original magnification, ×400). For each biomarker patient tumor sections are presented in order, for example panel A=Pt 1; panel B=Pt 2; panel C=Pt3; and panel D=Pt 4 and so forth for all 16 panels.

completing this therapy, a PET scan revealed findings consistent with local recurrence, a new active lesion in the left thyroid, and multiple metabolically active lesions in both lungs. After receiving chemotherapy with cetuximab/rapamycin, a follow-up PET scan showed a solitary primary lung lesion that was biopsied to confirm metastatic disease. There was no residual activity in the primary tumor site. She underwent resection of the lung lesion, in addition to cetuximab chemotherapy. She subsequently developed hoarseness and biopsies revealed squamous cell cancer of the anterior larynx. This likely represented a new primary as it developed remotely from her original primary but could have been a recurrence of her original tumor. This was treated with a laryngectomy. At the time of surgery, the hypermetabolic lesion in the thyroid was found to be a papillary thyroid cancer. She developed distant metastasis, was treated with palliative chemotherapy, and died several months later.

Patient 4. A 37-year-old white woman with a history of premalignant changes of the right lateral tongue, including numerous biopsies for dysplasia as well as CO_2 laser ablation for possible carcinoma in situ and leukoplakia. She became pregnant and noted that her tumor "took off" after the birth of her child. Shortly after she completed her pregnancy, she noticed a mucosal tear of the glossotonsillar sulcus following an episode of vomiting. One month later, she noticed an enlarging lump in the area of the right posterior tongue and a loose molar that was extracted. She had no history of tobacco or alcohol use, but her family history was positive for head and neck cancer in her grandfather. Pathology revealed SCC on the right lateral tongue and right retromolar trigone.

She underwent a right-sided composite resection of the lateral tongue, floor of mouth, and lateral mandible with a right selective neck dissection of levels I–IV. The primary tumor was a moderately differentiated, infiltrative 5.5-cm × 4.4-cm mass. Several lymph nodes were positive, but none exhibited extracapsular extension. This tumor was classified as T4aN2bM0 (Figure 1D) with a type 2 pattern of invasion.[10] She completed radiation therapy approximately 3 months later. As of her last follow-up visit, she has been free of disease 12 years from diagnosis.

Biomarker analysis

The patients' tumors had similar biomarker expression patterns. We used biomarkers that have been previously implicated in tumor behavior and response to therapy. All 4 tumors overexpressed epidermal growth factor recptor

TABLE 1. Summary of tumor characteristics and biomarker status in the 4 patients.

Characteristic/biomarker status	Patient 1	Patient 2	Patient 3	Patient 4
TNM classification	T4aN2bM0	T2N2bM0	T2N0M0	T4aN2bM0
Tobacco use (pk/y)	Yes (6)	No	No	No
Pattern of invasion	3	3	4	3
Perineural invasion	Yes	No	Yes	No
Tumor differentiation	Well	Moderate	Moderate	Moderate
Extracapsular spread	Yes	Yes	N/A	No
Delay in diagnosis (months)	7	0	10	Unknown
Breaks in treatment	Yes	No	No	No
EGFR overall IHC score*	16	16	16	12 (3, 4)
Bcl-xL overall IHC score*	12 (3, 4)	16	16	16
c-MET overall IHC score*	16	16	2 (2, 1)	12 (3, 4)
ER overall IHC score*	0	0	0	0
PR overall IHC score*	0	0	0	0
HER-2 overall IHC score*	0	0	0	0
p53 overall IHC score*	12 (3, 4)	0	16	0
p16 overall IHC score*	2 (2, 1)	0	2 (2, 1)	2 (2, 1)
HPV status	Negative	Negative	Negative	Negative
Survival outcome	Dead from disease	Alive, no evidence of disease	Dead from disease	Alive, no evidence of disease

Abbreviations: EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HPV, human papillomavirus; N/A, not applicable. *IHC score represents a product of a 4-point intensity scale (1 = no staining; 2 = weak; 3 = moderately strong; 4 = intense signal) and A 4-point proportion-positive scale where 1 $\leq$10%, 2 = 11%–25%; 3 = 26%–50%; 4 = 51%–100%. For scores less than 16, numbers in parentheses are (intensity score, proportion score).

(EGFR), all with an IHC score of 16 (Figures 2A–2D), and Bcl-xL, with an IHC score of 12 (3,4) for patient 1 and 16 for patients 2 to 4 (Figures 2E–2H). c-Met overexpression was observed in tumors from patients 1, 2, and 4, with IHC scores of 16 for patients 1 and 2 and 12 (3,4) for patient 4 (Figures 2I, 2J, and 2L) but not patient 3, with an IHC score of 2 (2,1) (Figure 2K). p53 was overexpressed in patients 1 and 3 only (Figures 2M, 2O). All 4 of the tumors were negative for p16, estrogen receptor, progestrone receptor, and HER-2 staining (not shown). Table 1 summarizes these biomarker results.

Etiology

Smoking and alcohol use were not a consistent factor in the etiology of these tumors. Only 1 patient admitted to a tobacco use history of 6 pack-years, and none admitted to alcohol abuse. Only 1 patient had a family history of head and neck cancer in a grandparent. All 4 tumors were also negative for high-risk HPV as assessed by a sensitive and accurate PCR mass spectroscopy assay[9] as well as by $p16^{INK4a}$ expression, a sensitive surrogate marker for transcriptionally active high-risk HPV involvement.[11] Except for the common factor of pregnancy, the etiology of these tumors remains a mystery.

DISCUSSION

Although HNSCC in pregnant patients is rare, it is important to understand the biology of these tumors to treat the cancer appropriately while minimizing risk to the fetus. Unfortunately, the increasing incidence of HNSCC in young patients means that likely there will be an increasing incidence of this disease in young pregnant women. The etiology of oral squamous cancer in young women in general, and particularly pregnant women, is perplexing. HNSCCs are typically the result of many years of tobacco and alcohol abuse, and have been predominantly a disease of men in their seventh and eighth decades of life who exhibit these social behaviors.[1,2] HNSCCs are rare in women, although with the increase in social acceptance of cigarette smoking during the 1940s and 1950s and active tobacco marketing campaigns for women in the 1960s and 1970s, the proportion of women with these cancers has been slowly rising such that instead of 4:1 male-to-female ratio the current ratio is closer to 3:1 or less.[3] Nevertheless, most women that develop head and neck cancers have long histories of exposure to tobacco smoke and are in the later decades of life when diagnosed. The four pregnant patients ranged in age from 26 to 37 years. Interestingly, a review of oral cavity cancer cases in our database revealed a total of 13 patients age 30 years and under. Remarkably, there was an inverted sex ratio, with a 11:2 ratio of females:males, including 2 of the pregnant women, both of whom died of their disease. In contrast, the remaining 8 of 11 females are currently alive with no evidence of disease. On the other hand, both males under 30 years died from disease within 16 months of diagnosis. Although the number of patients age 30 years and under is small, the predominance of females in this age group is striking, as is the better survival among the nonpregnant females in this age category. Thus, it is important to determine the factors associated with the development of these tumors in young females, and especially during pregnancy.

There were few clues to the differences in outcome among the pregnant patients. One factor we considered was whether any of these females experienced a delay in diagnosis. In fact, patient 1 initially presented with a sore on her tongue that was thought to be a chancre sore and was subsequently treated with antibiotics 6 months prior to diagnosis of malignancy. Patient 3 noted an intermittent soreness that waxed and waned for 10 months before the pain became severe enough for her to seek medical opinion. Patient 4 had a long history of a dysplastic lesion

that was being closely monitored and thus was diagnosed when it seemed to accelerate growth after pregnancy. It is unclear whether diagnosis was delayed in patient 2, as she was initially seen and diagnosed at a different institution and complete records were not available.

Pregnancy may induce physiologic changes that can promote neoplastic growth, such as a high metabolic state, increased circulating growth factors, and amplified hormonal responses mediated through the estrogen and progesterone receptors.[4,12,13] Increased expression of ER and PR have been linked to several types of cancers. The binding of the respective ligands to ER and PR stimulates the proliferation of certain cell types, which may enable DNA mutations to persist as a result of increased cell division.[14] Because these patients were pregnant, it seemed plausible that the high levels of estrogen and progesterone circulating during pregnancy would contribute to oncogenesis if the malignant cells expressed ER and/or PR. Some studies have shown an absence of both estrogen and progesterone receptors in SCC cells.[15] However, others have reported that the majority of cases of HNSCC in males overexpress these receptors, and the highest tumor-free survival in that study was in the ER(+)/PR(−) group.[16] Our results in the present study fail to implicate expression of either ER-α or PR in these tumors.

HER-2 is a cell membrane–bound tyrosine kinase that is involved in a signal transduction pathway that leads to cell proliferation. HER-2 is an important biomarker in breast cancer, and targeting HER-2 with herceptin is an important component of breast cancer therapy. Recently, some studies have explored HER-2 expression in HNSCC[17,18] but were inconclusive with respect to its effect on prognosis. Because of the altered hormonal milieu during pregnancy, we assessed HER-2 expression in these oral cancers as a possible biomarker. However, all of the tumors were HER-2 negative. In the case of breast cancers, HER-2 in combination with ER-α and PR is used as an indicator of prognosis.[19] Triple-negative breast cancers (negative for ER-α, PR, and HER-2) are known to have a low response to drug therapy and a worse prognosis.[20] Although our patients' tumors were all triple negative for these markers, in the absence of a population of oral tumors that express these markers, it is not possible to ascribe any significance to this negative finding.

The TNM classification system is used for assessing the tumor stage of HNSCC. This system is based on primary tumor size, regional lymph node involvement, and distant metastases as variables for staging. Although tumor classification can help to determine prognosis, recent studies have demonstrated that more specific histopathologic markers play a significant role in assessing prognosis. Among these biomarkers are perineural invasion and pattern of invasion. Perineural invasion has been associated with poor prognosis in many cancers, including HNSCC.[21] Because of its association with tumor aggressiveness, it may be 1 of the most significant tumor characteristics influencing survival,[21,22] and local recurrence.[23] Patients 1 and 3 had tumors that exhibited perineural invasion. Both of these patients experienced aggressive disease progression with multiple recurrences and death from their disease compared with patients 2 and 4, whose tumors lacked perineural invasion. Our results are consistent with perineural invasion as an indicator of poor prognosis.

Another histopathologic biomarker that has been shown to influence prognosis is the pattern of invasion. Pattern of invasion (POI) is defined on a scale from type 1 to 4. A POI type 1 is characterized by broad, pushing borders at the invasive front; type 2 indicates a tumor with broad, invasive fingers (separate large tumor islands); type 3 is characterized by smaller invasive islands with more than 15 cells per island; and a POI type 4 is the most invasive type, defined as containing invasive tumor islands smaller than 15 cells per island.[10] It has been shown that using this histopathologic assessment over margin status is more predictive of local disease-free and overall survival.[24] Patient 4 with POI 2 is a long-term survivor, whereas only 1 of the 3 patients with more invasive POI has survived long term without disease recurrence. Patient 3 with the most invasive POI, type 4, experienced significant disease progression including multiple distant metastases.

EGFR is a receptor tyrosine kinase that plays a role in several physiologic and pathologic processes, including apoptosis, cell cycle progression, and metastasis. It is commonly upregulated in many cancers, especially HNSCC.[29,30] Overexpression of EGFR in oropharyngeal cancer is inversely associated with response to induction chemotherapy and chemoradiation therapy, and thus it is directly associated with a poor prognosis.[8] In HPV(−) oropharyngeal tumors, we found that the tumors with EGFR overexpression led to a poor prognosis, and all HPV-negative patients in a recent organ sparing trial with the EGFR-high phenotype died of their disease within 2 years of diagnosis.[8] In the HPV(+) cases in the same trial, high expression of EGFR was associated with significantly reduced disease-specific and overall survival when compared with HPV(+)/EGFR(−) cases. In spite of the high EGFR/HPV(−) phenotype of all 4 tumors, 2 of these 4 patients have enjoyed long disease-free survival.

The tumor suppressor p53 is a key component of cell cycle regulation. In the setting of DNA damage, p53 induces cell cycle arrest and DNA repair mechanisms. Mutations in the p53 gene have been implicated in numerous cancers[8,31] and are particularly common in smoking-induced head and neck cancers. Wild-type p53 is expressed only transiently and, therefore, it typically cannot be detected by immunohistology in normal cells. Tumors that stain positive for p53 typically have a mutation that allows p53 to accumulate within the cell.[32] Although p53 is often mutated in oral cancers, in tumors induced by HPV, the HPV E6 protein causes degradation of p53, inactivating it. Thus, p53 is typically wild-type in HPV-induced cancers, but wild-type p53 is uncommon in HPV-negative HNSCC.

We found low or no expression of p53 in 2 of the tumor specimens, consistent with wild-type p53 in these patients; however, both patients whose tumors progressed overexpressed p53, consistent with mutant p53. We previously had established a cell line from patient 1 and in the course of characterizing the line we sequenced the full-length cDNA for p53. This revealed a point mutation at codon 157 that substitutes alanine for valine (V157A). This is consistent with the high expression of p53 in the

patient's tumor. Although we have not sequenced p53 in the tumor from patient 3, the high expression indicates a mutation in that tumor as well.

High-risk human papillomaviruses (HPVs), especially HPV16, are now clearly implicated as etiologic factors in many oropharyngeal and some oral cavity cancers.[25,26] HPV-positive oropharyngeal cancers are also associated with younger age at diagnosis and may arise in people without a history of tobacco use. Furthermore, HPV-positive oropharyngeal cancers are more likely to respond to induction chemotherapy and chemoradiation therapy.[8,9] $p16^{INK4a}$ is a cyclin-dependent kinase inhibitor that blocks cell cycle progression by inhibiting pRb phosphorylation.[27] Whereas silencing of $p16^{INK4a}$ is common in many smoking-related head and neck cancers,[28] it is nearly always overexpressed in HPV-positive tumors secondary to the effect of HPV E7 oncoprotein sequestering of Rb, resulting in release of E2F, and upregulation p16 expression. Thus, immunohistology for p16 is often used as a marker of HPV expression.[11] Three tumors expressed only low-intensity staining of p16 in a minority of cells, which is in contrast to the high p16 intensity and 100% tumor cell positivity that is typically observed in HPV-positive tumors. The lack of robust p16 staining was confirmed by quantitative real-time PCR and MALDI-TOF mass spectroscopy,[9] which failed to identify any high-risk HPV in the tumor specimens. Thus, we can eliminate HPV as a likely etiologic factor to explain the development of these tumors at such an early age. However, the absence of HPV in all 4 of these tongue cancers arising in nonsmokers is perplexing. It suggests that there is an unusual mechanism of carcinogenesis, or perhaps that even an inherited predisposition to cancer development is at work in these young pregnant females. High throughput assessment of the full cancer genome in each of these tumors may produce insight into the carcinogenic mechanism.

Bcl-xL, an anti-apoptotic protein, is frequently overexpressed in HNSCCs.[8,32,33] Bcl-xL contributes to tumorigenesis and treatment resistance by enabling cells that have been damaged by carcinogens, chemotherapeutics, or radiation to evade apoptosis. In laryngeal cancer, Bcl-xL overexpression in combination with low expression of wild-type p53 is associated with a decrease in response to chemotherapy and a 16-fold increased risk of laryngectomy.[34] The combination of low p53 and high Bcl-xL levels is also associated with decreased overall survival in oropharyngeal cancer.[8] Bcl-xL was overexpressed, and p53 was low or undetectable in the pregnant patients, suggesting that chemotherapy would be unlikely to add significant benefit. Interestingly, both patients who received chemotherapy failed to exhibit a strong benefit.

c-Met overexpression is highly correlated with tumor invasiveness and poor overall survival in multiple cancer types.[35–37] c-Met inhibitors are being developed that decrease metastatic growth and invasiveness in several types of cancer.[38–40] Although the data implicating c-Met specifically in HNSCCs are sparse, the data that do exist indicate that it is an important biomarker of poor prognosis and outcome in this disease. Interestingly, patient 3 had a tumor with a pattern of invasion type 4, the most invasive type. This patient was the only of the 4 that had an aberrant pattern of c-Met expression and did not floridly overexpress c-Met. Clearly more work on the association between c-Met and pattern of invasion as well as the basis for aberrant c-Met expression in oral cancer requires more study. Similarly, the presence of c-Met overexpression in 3 of the 4 tumors from pregnant patients suggests that the use of c-Met inhibitors may have a therapeutic role in these cancers.

Although the biomarker expression pattern is similar among all 4 patients in this study, disease progression and survival vary greatly. Two patients, 2 and 4, had completed their pregnancy by the time their cancer was diagnosed and treated. It is possible that critical hormonal influences had diminished in these patients after they completed their pregnancy, allowing for their better outcomes. The lack of ER and PR expression does not exclude the possibility of other hormonal influences impacting the growth and spread of these cancers, given that estrogen and progesterone are not the only hormonal variables that change dramatically during pregnancy. For example, growth factors such as insulin-like growth factor (IGF-1) and others that are elevated during pregnancy could have a potent influence on epithelial cell proliferation and tumor progression. Further study is required to determine this possibility.

Patients 2 and 4 were able to undergo surgery followed by radiation because they had both delivered their babies. These 2 patients are alive and well more than 12 years after diagnosis. Patients 1 and 3 both exhibited extensive perineural invasion, a known poor prognostic factor in head and neck cancer, and patient 3 exhibited the most aggressive POI. These findings prompted consideration for adjuvant therapy.

Patient 1 had interrupted postoperative radiation and chemotherapy and returned to complete her treatment months after her initial therapy and after her recurrent cancer was inoperable. It is known that prolonged treatment breaks can affect ultimate local and regional control rates and prognosis. Although this patient chose to terminate her pregnancy, her inability to complete the recommended course of therapy may have contributed to her poor outcome.

Patient 3 continued her pregnancy and did not receive radiation or chemotherapy postoperatively, as she had no detectable nodal spread, and her only concerning risk factor was perineural invasion. It is unclear whether radiation with or without chemotherapy improves local and regional control rates or overall survival in patients whose tumors exhibit perineural invasion. The NCCN guidelines suggest considering radiation or radiation with chemotherapy for patients whose tumors exhibit perineural invasion, but so far there is no study that indicates that this added therapy improves prognosis. Given the uncertainty that additional treatment would prove to be beneficial, the patient chose to continue her pregnancy. This patient's cancer behaved in a very aggressive manner and the influence of aberrant c-Met expression on her tumor biology warrants further investigation.

Pregnancy complicates the management of patients. Pregnancy is a state of relative immunosuppression and increased metabolic and hormonal activity that may affect the outcome of treatment for a wide variety of diseases. Any treatment that is chosen will affect both the mother and the fetus. The physician and mother must try

to balance the need for treatment and the risks to the fetus. Whenever possible, if equivalent therapies are available, the one with the least risk to the patient and fetus should be selected. Treatment decisions are much more difficult when the efficacy of the treatment is unclear and the risks may be significant, as is the case with perineural invasion in head and neck cancer. The decision to terminate a pregnancy is generally accepted to be a personal choice. The treating physician has an obligation to advise the mother regarding treatment options, expected effects, and risks to the fetus. The mother ultimately decides with the help of this advice and that from other individuals; then she may choose to continue the pregnancy or not.

Physicians and expectant mothers may have a tendency to avoid therapy or postpone full treatment until after the completion of pregnancy for fear of causing harm to the fetus. Ideally, treatments that are highly effective and have little or no risk to the fetus would be available. New treatments for HNSCC have begun to focus on the significance of molecular inhibitors that silence specific molecular pathways that may decrease the severity of side effects while increasing the response to therapy. Administration of a more specific treatment regimen that is tailored to the specific tumor biology would be more effective and decrease the toxicity to the patient. This concept is particularly important in pregnant women to minimize harm to the fetus while treating the cancer appropriately. Further investigation is necessary to help determine the exact biology of these tumors to facilitate a more specific treatment regimen that will balance both maternal and fetal health.

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