Vol. 118 No. 3 September 2014

Oral squamous cell carcinoma positive for p16/human papilloma virus in post allogeneic stem cell transplantation: 2 cases and review of the literature

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Complications of allogeneic hematopoietic stem cell transplantation (allo-HSCT) includes the risk of secondary malignancies. This may be related to mechanisms including radiation and chemotherapy regimens, chronic graft-versus-host disease, inflammation, and prolonged imunosuppression. Oral squamous cell carcinoma (OSCC) is a complication associated with chronic graft-versus-host disease after allo-HSCT. Although human papillomavirus (HPV) is known to be associated with OSCC, the role of HPV in development of OSCC in post-HSCT patients has not been studied. We identified 2 cases of OSCC in allo-HSCT recipients. Both biopsy specimens tested positive for $p16^{INK4A}$, a surrogate marker for HPV. We propose that the association of OSCC and HPV in patients after allo-HSCT may not be incidental. Clinical implications of these cases may imply the need for a HPV screening, early intervention, and consideration of anti-HPV vaccination in this population. The effectiveness of such interventions could be validated in a prospective clinical study. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;118:e74-e78)

With improved outcomes after allogeneic hematopoietic stem cell transplantation (allo-HSCT), increasing attention has been drawn to late complications in long-term survivors. Among these, survivors of allo-HSCT are at significantly increased risk for developing secondary malignancies, with the incidence of secondary solid tumors 2% to 6% at 10 years and 6% to 13% at 15 years.[1-4] Squamous cell carcinoma (SCC) of the skin and mouth is the most common secondary solid malignancy, accounting for one third of all secondary solid tumors, with oral SCC (OSCC) representing 50% of these cases.[1,2,5] In one of the largest studies, Curtis et al.[1] analyzed 19,229 allo-HSCT patients from 235 centers and found that chronic graft-versus-host disease (cGVHD) and male sex were strongly associated with increased risk of OSCC.[1] In a large multicenter European study, the actuarial risk of second malignances at 15 years was 11.5 ± 2.3%.[4] Furthermore, higher doses of total body irradiation were associated with higher incidence of solid tumors.[1] Another larger study by Rizzo et al. on 28,000 patients found that the risk of SCC was 5 times higher in patients with cGVHD than the general population.[6] Bhatia et al. reported that 25% late mortality in allo-HSCT survivors was attributed to treatment-related causes, including 7% from secondary malignancies.[7] Patients with Fanconi's anemia were reported to have higher incidence of head and neck squamous cell carcinoma (HNSCC) with poor survival.[8,9]

Possible mechanisms that have been proposed include radiation mutagenesis, cGVHD-related inflammation, prolonged immunosuppression for cGVHD, immunologic dysfunction, and carcinogenic and cytotoxic effects of immunosuppressive therapy, or a combination thereof.[5,6,10] Post-HSCT OSCC has been reported to occur in unusual sites such as the vermillion border of the upper lip and the midline dorsum of the tongue, buccal mucosa, or gingiva.[11-13]

The association of human papillomavirus (HPV) and SCC of the oral cavity, female genital tract, and skin is well established in the general population, and immunosuppressed patients such as patients with human immunodeficiency virus (HIV), patients who have undergone cancer chemotherapy, and those who are solid organ transplant recipients. HPV involvement in OSCC was first suggested by Syrjanen K et al.[14] and confirmed by multiple studies thereafter.[15-22] HPV has been associated with OSCC diagnosed in the general population in about one third of cases.[19-22] In a published meta-analysis of 5,046 head and neck SCC cancer specimens from 60 studies, the overall HPV prevalence was 25.9%; however, HPV prevalence was significantly higher in oropharyngeal SCC (35.6%). HPV16 accounted for the majority of HPV-positive

Statement of Clinical Relevance

Screening for high risk human papilloma virus could be considered in post allogeneic hematopoietic stem cell transplantation patients with lesions suspicious for oral carcinoma.

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Received for publication Apr 23, 2014; returned for revision May 20, 2014; accepted for publication May 31, 2014.

2212-4403
http://dx.doi.org/10.1016/j.oooo.2014.05.025

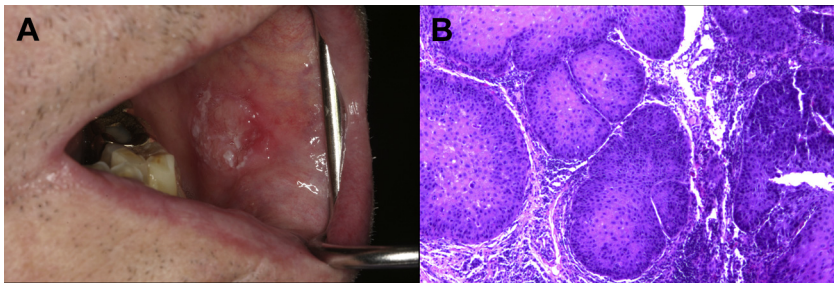

Fig. 1. **(A)** Clinical photograph from Case1 demonstrating a speckled red and white lesion of the buccal mucosa. **(B)** Photomicrograph from Case 1 demonstrating atypical bulbous proliferations and islands of neoplastic epithelium invading into the connective tissue (hematoxylin-eosin, original magnification ×50).

oropharyngeal SCCs (86.7%).[23] Although other oncogenic HPVs were detected in HNSCC, in a recent case-controlled study HPV16 DNA predominated, occurring in 72% of 100 paraffin-embedded tumor specimens of oropharyngeal cancer.[17]

The contribution of HPV to the higher incidence of OSCC in allo-HSCT long-term survivors has not been well defined. In a small study, HPV was detected in 3 out of 5 cases of post-transplant OSCC.[24] Three of these patients had undergone allo-HSCT and had cGVHD. In another study from Taiwan, half of the allo-HSCT patients with secondary carcinoma had oral squamous cell carcinoma; however, none of the tumors had HPV infection according to immunohistochemistry (IHC) staining.[25] Special groups of patients who tend to have high incidence of HNSCC after allo-HSCT are the patients with Fanconi's anemia and aplastic anemia.[8,26] These patients and patients with severe aplastic anemia who undergo allo-HSCT were reported to have a higher incidence of oral HPV, especially high-risk HPV16, in comparison to healthy patients.[27] Overall, the reports about HPV involvement in OSCC after HSCT are rather scant but may have a profound clinical significance.

An improved understanding of the clinical features and potential factors associated with secondary OSCC, as well as its course and treatment outcomes, may be beneficial in better understanding, predicting, and managing this very serious late toxicity of allo-HSCT. The objective of this report is to describe 2 cases of OSSC after allo-HSCT that occurred in unusual sites and in patients who were also positive for HPV.

CASE REPORTS

Case 1

A 68-year-old man, a nonsmoker, was referred to the oral medicine clinic for a nonhealing ulcer on the left buccal mucosa. The patient's medical history was significant for intermediate grade large B-cell lymphoma that was treated 10 years ago by nonmyeloablative allo-HSCT, preceded by 200 cGy total body radiation in 1 fraction. The patient had a history of skin and oral cGVHD. He had a relapse of his lymphoma isolated to the parotid and received rituximab for 2 years. He also has had monthly treatment with intravenous immunoglobulin because of hypogammaglobulinemia and recurrent infections but has maintained clinical remission of his lymphoma since then.

Extra oral examination revealed a firm 1 cm lymph node on the left inferior border of the mandible. The intraoral examination revealed a 1.5 cm × 1 cm raised white ulcerated lesion on the left buccal mucosa (Figure 1, A) with distinct borders and speckled erythro-leukoplakia. The borders of the lesion were firm and indurated. An incisional biopsy was performed revealing large epithelial islands and atypical bulbous extensions of neoplastic epithelium invading the underlying the fibrous connective tissue. The neoplastic epithelium displayed significant cytologic and morphologic alterations, including increased nuclear/cytoplasmic ratios, nuclear hyperchromatism, individual cell keratinization, abnormal mitoses, and formation of squamous eddies (Figure 1, B). A diagnosis of superficially invasive squamous cell carcinoma stage T_1 M_0 N_1 was rendered. The patient underwent a wide local excision and deep neck dissection with postoperative adjuvant radiation therapy.

Case 2

Case 2 was an 18-year-old male nonsmoker with acute myeloid leukemia for which he had allo-HSCT from unrelated matched donor. The patient had a history of cGVHD on the lower lip; he also had a fair complexion and a history of sun exposure. Nine years after allo-HSCT, he developed a crusty lesion on the vermillion border of the upper lip that progressed into an indurated nodule (Figure 2, A). An incisional biopsy of the lesion revealed widely invasive well-differentiated squamous cell carcinoma with formation of numerous islands and clusters of neoplastic cells. Extensive keratin pearl formation along with nuclear and cellular pleomorphism was noted throughout the specimen (Figure 2, B). The malignancy was staged at T_1 M_0 N_0. The patient's upper lip was subsequently treated with radiotherapy and the cancer did not recur.

Laboratory studies

Four μm thick sections were prepared from formalin-fixed paraffin-embedded tissue from cases 1 and 2 for IHC staining with monoclonal antibodies against the tumor-suppressor

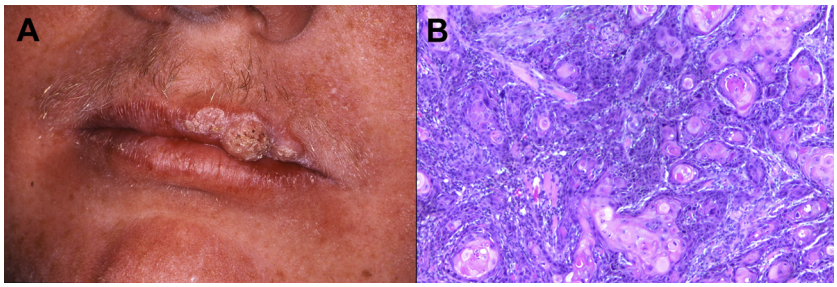

Fig. 2. **(A)** Clinical photograph from Case 2 depicting a nodular, ulcerated, and raised, rough, indurated lesion of the upper lip. **(B)** Photomicrograph from Case 2 shows a widely invasive proliferation composed of small islands and clusters of malignant epithelium with numerous keratin pearls and foci of individual cell keratinization (hematoxylin-eosin, original magnification ×50).

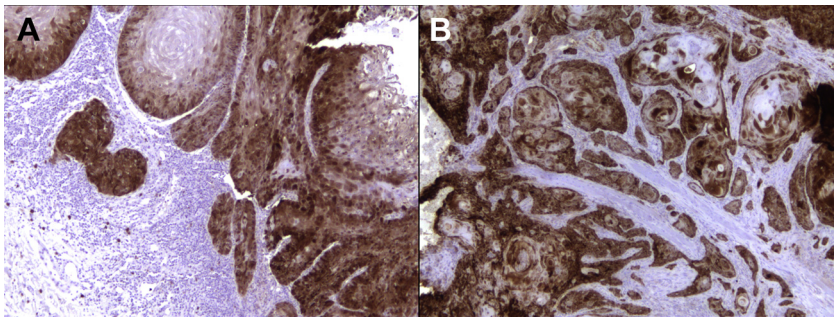

Fig. 3. Photomicrograph of immunohistochemical stain for $p16^{INK4A}$ in the biopsy sample of Case 2 demonstrating strong positive staining in majority of the invasive portions of the malignant process. **(A)** Bulbous extensions of neoplastic epithelium are noted. **(B)** The neoplasm is more widespread and smaller islands with keratinization are noted throughout the stromal connective tissue (original magnification ×50).

gene $p16^{INK4A}$ considered a surrogate marker for high-risk HPV infection.[28,29] Sections were subsequently stained using a Dako Autostainer Plus system (DAKO, DakoCytomation, Glostrup, Denmark). Endogenous peroxidase activity was quenched by incubating the slides in peroxidase blocking reagent for 10 minutes. Incubation with mouse anti-human $p16^{INK4A}$ monoclonal antibody (Santa Cruz Biotechnology, Dallas, TX) (diluted 1:100) was performed for 30 minutes at room temperature. Sites of binding were detected using 3,3′-diaminobenzidine as chromogen according to the manufacturer's instructions. The sections were counterstained with hematoxylin, dehydrated, cleared, and mounted. The sections from both specimen were strongly reactive for $p16^{INK4A}$ (Figure 3).

DISCUSSION

In this report, we describe 2 p16/HPV-positive OSCC cases; both patients are males and 1 is young, 18 years old. It has been known previously that there higher prevalence of OSCC in males and that it can happen in younger age in immunocompromised patients. This report also brings up multiple important points that can have clinical implications to the diagnosis, treatment, and follow up of surviving patients with cGVHD after allo-HSCT, including the following: Secondary OSCC is a serious complication of these patients; HPV may play a role in the pathogenesis of such complication; and testing for HPV by IHC for p16 is simple and should be done on all patients with OSCC. These issues may have wider application in patients who receive intense chemotherapy and in patients undergoing solid organ transplants.

Many questions remain, including whether the detection of HPV represents a new infection of HPV or a reactivation of a latent infection. Reactivation of latent DNA viruses such as herpes simplex, varicella zoster, human herpes virus 6, Epstein-Barr, cytomegalovirus, and hepatitis B virus is known to happen after allo-HSCT.[30] Although HPV infection is common, studies suggest approximately 90% of infections clear within 2 years.[31] However, many patients in whom HPV has been eliminated from the serum still have detectable HPV DNA in tissues such as skin, in the oral cavity, and in the female genital tract. Thus, after allo-HSCT, renal transplantation, or intensive chemotherapy dormant HPV may reactivate in these sites, leading to benign and malignant tumors.[31] A meta-analysis by Grulich et al.[32] indicated that HIV-infected patients and solid organ transplant recipients had an increased incidence of HPV-related cancers, including the genitalia and oropharynx. HPV DNA was shown in the oral

cavity (brushings collected and used for consensus polymerase chain reaction) of 18% of renal transplant versus 1% of control samples.[33]

Studies done on HPV vaccination of high-risk females have shed light on the immune response against HPV. Natural immunity against HPV involves innate and adaptive immune responses, predominated by a robust, local cell-mediated immunity, which is associated with lesion regression and generation of serum neutralizing antibodies.[34,35] However, clearance of HPV infection is diminished among immunocompromised individuals, possibly leading to reactivation and progression to malignant lesions.[36,37] Furthermore, there is evidence that seroreactivity against HPV can be lost after allo-HSCT.[38] This may imply that these patients may acquire the infection after HSCT. Thus the need for HPV vaccination after allo-HSCT has been discussed before.[30,39] The timing and the population target for such vaccination should be studied in a prospective large study that may take many years to complete.

In conclusion, we present 2 cases of post−allo-HSCT OSCCs in patients who were positive for p16 HPV, thus raising the issue of risk of HPV-related OSCC with clinical implications for screening and early intervention. A large cohort study is warranted to formulate distinct clinical recommendation; however, patients at risk, mainly long-term survivors with cGVHD, should probably be screened for HPV using IHC or polymerase chain reaction on oral brushings and if positive be carefully and closely monitored for secondary OSCC for early intervention and prevention.

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