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Chrysotile fibers in tissue adjacent to laryngeal squamous cell carcinoma in cases with a history of occupational asbestos exposure

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Abstract

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DISCLOSURE/CONFLICT OF INTEREST

The authors have no conflicts to disclose.

Asbestos describes a group of naturally occurring fibrous silicate mineral compounds that have been associated with a number of respiratory maladies, including mesothelioma and lung cancer. Additionally, based primarily on epidemiologic studies, asbestos has been implicated as a risk factor for laryngeal and pharyngeal squamous cell carcinoma (SCC). The chief objective of this work was to strengthen existing evidence via empirical demonstration of persistent asbestos fibers embedded in the tissue surrounding laryngeal and pharyngeal SCC, thus providing a more definitive biological link between exposure and disease. Six human papillomavirus (HPV)-negative laryngeal (n = 4) and pharyngeal (n = 2) SCC cases with a history working in an asbestos-exposed occupation were selected from a large population-based case-control study of head and neck cancer. A laryngeal SCC case with no history of occupational asbestos exposure was included as a control. Tissue cores were obtained from adjacent non-neoplastic tissue in tumor blocks from the initial primary tumor resection, and mineral fiber analysis was performed using a scanning electron microscope equipped with an energy dispersive x-ray analyzer (EDXA). Chrysotile asbestos fiber bundles were identified in 3/6 of evaluated cases with a history of occupational asbestos exposure. All 3 cases had tumors originating in the larynx. In addition, a wollastonite fiber of unclear significance was identified one of the HPV-negative pharyngeal SCC cases. No mineral fibers were identified in the adjacent tissue of a case without occupational exposure. The presence of asbestos fibers in epithelial tissue surrounding laryngeal SCC in cases with a history of occupational asbestos exposure adds a key line of physical evidence implicating asbestos as an etiologic factor.

Keywords

Head and neck cancer; laryngeal cancer; occupational cancer; fiber count analysis; wollastonite

INTRODUCTION

According to the most recent global cancer statistics, pharyngeal & laryngeal cancer accounted for an estimated 350,917 global incident cancer diagnoses and 180,770 deaths in 2018 (1). Of these, more than 90% are squamous cell carcinoma (SCC) (2), with smoking, alcohol consumption and human papillomavirus (HPV) infection, particularly for oropharyngeal SCC, being widely recognized as the primary risk factors (3). While those risk factors have garnered the majority of attention in etiologic research – deservedly so since they are attributed to the vast majority of these tumors – there is evidence that occupational exposures are also risk factors (4, 5).

Asbestos describes a group of naturally occurring fibrous silicate mineral compounds. Its use dates back thousands of years, but became wide-spread in the late 19th century through the late 20th century (6) due to its favorable industrial properties, including its strength, flexibility and thermal properties, with peak use in the United States occurring in the 1970s. Asbestos has been associated with a number of chronic respiratory diseases (7), including malignancy, and was linked to mesothelioma (8, 9) and lung cancer (10) in the early to mid-20th century. Despite the known health risks, asbestos is not universally banned, and is still in use in many countries (including the United States), with an estimated 125 million occupationally-exposed individuals worldwide (11).

In addition to mesothelioma and lung cancer, asbestos has also been implicated as a risk factor for cancers involving the upper airway, in particular laryngeal and pharyngeal SCC (4, 12–14); however, the evidence thus far has been primarily based on epidemiologic data from observational studies. Therefore, the goal of this study was to strengthen the existing evidence implicating asbestos as a risk factor for pharyngeal and/or laryngeal SCC via confirmation of persistent asbestos fibers in epithelial tissue surrounding laryngeal and pharyngeal SCC in cases with a history of occupational asbestos exposure.

MATERIALS & METHODS

Study Population

Six cases with incident, HPV-negative pharyngeal (n = 2) and laryngeal (n = 4) SCC (ICD-9: 146, 148, 149.0, 149.1, and 161) with self-reported occupational exposure to asbestos were selected from a large population-based case-control study of head and neck cancer, plus one laryngeal SCC case with no occupational exposure history as a control, matched to the sex, race/ethnicity, and median age (+/- 3 years) and smoking pack-years (+/- 3 pack-years) of the asbestos-exposed cases. Briefly, detailed occupational history was available for 1,056 head and neck cancer cases enrolled through major teaching hospitals located in Boston, Massachusetts (15–17). Occupational history was systematically reviewed to verify that cases with self-reported occupational history worked in an occupation at high risk for asbestos exposure. All cases selected had archival tumor and adjacent normal tissue available at Massachusetts General Hospital.

High-Risk HPV Testing—Serologic testing was performed for antibodies against E6 and E7 viral oncoproteins for high-risk HPV (HPV 16, 18, 31, 33, 45, 52 and 58) using sandwich ELISA assays, as previously described (18–22). Subjects were considered high-risk HPV seropositive if antibodies were present for either viral oncoprotein.

Asbestos Fiber Counts

Cores were obtained from adjacent non-neoplastic tissue in tumor blocks from the initial primary tumor resection. A count of asbestos fibers $\geq 5\mu\text{m}$ was performed on the cores from each case at Duke University via scanning electron microscopy (23). Specimens were deparaffinized in xylene, rehydrated in 95% ethanol, and wet weight was obtained on an analytical balance. The tissue was then digested in sodium hypochlorite solution and the residue collected on 13mm Nuclepore filters with a pore size of 0.4 μm . These were mounted on a carbon stub with colloidal graphite, sputter-coated with platinum, and examined by scanning electron microscopy (SEM) equipped with an energy dispersive x-ray analyzer (EDXA). One hundred consecutive fields were examined at a screen magnification of 1300x and all fibers $\geq 5\mu\text{m}$ in length counted and examined by EDXA (23).

Immune Assessment

For descriptive purposes, morphologic analysis was performed to assess severity and type of inflammation in the tumor microenvironment. “Global” qualitative assessments of inflammation severity were performed by two board-certified Anatomic Pathologists (each blinded to the other’s assessments), as well as assessments of the presence or absence of

dense lymphoid tissue and neutrophilic inflammation. The lymphoid infiltrate in each case was assigned one of three “patterns” depending on whether lymphoid tissue around tumor cells formed a continuous and dense rim (*Pattern 1*), was present as discontinuous dense patches (*Pattern 2*), or was limited or absent (*Pattern 3*) (24). Neutrophilic inflammation and global qualitative inflammation assessments were also scored as three-tiered variables (0–25%, 25–50%, >50%) and as mild, moderate and severe, respectively (25).

RESULTS

Study subject characteristics, including occupation(s) and duration spent working in an occupation with asbestos exposure, are presented in Table 1. Briefly, all asbestos-exposed cases were Caucasian males with a median age of 63.5 years (range: 56–77 years). Asbestos-exposed occupations/industries of the cases included automotive mechanic, space shuttle thermal protection applicator (solid-fuel boosters contained asbestos materials (26)), firefighter, Army artillery, school custodian, and metal lather, with a median duration spent in an asbestos-exposed occupation of 34.5 years (range: 5–56 years). The non-asbestos exposed case was a 64 years old Caucasian male with an occupational history as a truck driver. With the exception of one of the cases (HN2), all subjects had a smoking history, with a median of 39 packs-years (range: 0–114 pack-years). The reported occupational asbestos exposures for all cases overlapped peak industrial usage in the United States (27) as depicted in Figure 1.

Histopathologic slides were available for the primary tumors of 4/6 cases (Figure 2). Of those, two distinct patterns of inflammation were noted. Two cases showed prominent lymphoid infiltrates, which were present as a continuous dense rim in one case (HN1) and as discontinuous dense patches in the other (HN6), with each exhibiting minimal to absent neutrophilic inflammation. In contradistinction, the other two cases (HN3 and HN4) contained limited or no lymphoid tissue but did show prominent neutrophilic inflammation.

Chrysotile asbestos fiber bundles were identified by SEM in adjacent non-neoplastic tissue from 3/6 asbestos-exposed cases with spectra confirmed by EDXA, all of which had laryngeal SCC (Figure 3); neither of the two HPV-negative pharyngeal cancer cases had detectable asbestos fibers. Fibers ranged from 7–14 μm in length and 0.2–0.5 μm in diameter. It should be noted that, given the archival nature of the tissue, samples for all cases were well below the recommended amount of 0.3 g wet weight for fiber analysis (Table 2). A wollastonite fiber (calcium silicate) was identified in adjacent tissue in one of the two HPV-negative oropharyngeal cases (HN4), although this is of uncertain significance. No mineral fibers were identified in the adjacent tissue of the case without any known occupational exposure (control).

DISCUSSION

We have identified chrysotile asbestos fibers in non-neoplastic epithelial tissue adjacent to laryngeal SCC in cases with a history of work in an asbestos-exposed occupation, greatly strengthening the causal evidence for asbestos as a risk factor. Up to now, there had only been one other case report identifying mostly amphibole asbestos fibers in non-neoplastic

laryngeal tissue in a case with laryngeal SCC (28). The present work significantly adds to these previous reports, quadrupling the number of reported laryngeal SCC cases with detectable asbestos fibers and, importantly represents the first study to identify chrysotile asbestos fibers proximal to laryngeal SCC tumors in the absence of amphibole fibers.

These findings are in agreement with and complementary to existing epidemiological evidence from industrial cohorts and observational studies supporting an association of asbestos exposure and laryngeal SCC (12, 14). Our observations are also in-line with that from two separate reports of asbestos bodies and/or fibers in laryngeal tissue at autopsy from former asbestos workers with no detectable laryngeal cancer (28, 29). Of particular interest in our present study is the identification of chrysotile fibers in tissue from a laryngeal SCC case who identified as a never-smoker and moderate alcohol user – hardly the prototypical profile of a laryngeal cancer case – but with a high-risk occupational history working as a space shuttle thermal protection system applicator in the 1970s and over two decades as a firefighter (HN2).

Chrysotile fibers were only identified in asbestos-exposed laryngeal SCC cases, with none identified in tissue from either HPV-negative pharyngeal SCC case. However, it bears to be reiterated that the samples in this study ranged 2.7- to 3000-fold below the recommended 0.3 g of wet tissue weight that would be used with larger autopsy samples, and therefore samples with no identifiable asbestos fibers should be considered inconclusive rather than negative. To this point, one of the two pharyngeal cases (HN5) had the least amount of tissue available of all of the samples. Wollastonite – an acicular calcium silicate mineral fiber – was identified in the other of the two HPV-negative oropharyngeal cases (HN5). However the significance of this is uncertain, as an IARC working group determined two decades ago that wollastonite *cannot be classified as to its carcinogenicity to humans* due to insufficient human or experimental models (30) and little to no evidence to the contrary has been surfaced since (31).

There were several strengths to our study, notably use of SEM for fiber analysis with confirmation by EDXA spectra; detailed occupational history and other major risk factor data for the cases; and availability of adjacent non-neoplastic epithelial tissue, which allowed us to avoid a dilution effect that would be encountered by using tumor tissue due to clonal expansion. However, there were also some limitations. Due to labor and resource constraints, including availability of archival tissue materials, we were only able to evaluate a small subset of cases with occupational asbestos exposure in the parent study (17). However, despite the small sample size, it is notable that chrysotile fibers were identified in 3/6 of the cases interrogated, and were absent from the cases lacking an occupational asbestos history. Moreover, as we had previously stated, the wet tissue weight for each case was suboptimal for fiber detection, and therefore we cannot rule out the presence of fibers in pharyngeal or laryngeal tissue from the negative cases. Nonetheless, we still were able to identify fibers in a sizeable fraction of interrogated cases.

Despite its known adverse health effects, asbestos continues to be a threat to global health. Although asbestos has been fully banned more than 50 countries, it is still in widespread use among developing nations, and is notably still in use – albeit limited – in the United States

(32, 33). Our identification of chrysotile asbestos fibers in epithelial tissue proximal laryngeal SCC adds a critical empirical link and bolsters the mounting evidence that asbestos is a risk factor for laryngeal cancer.

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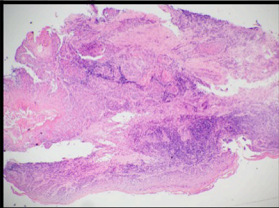
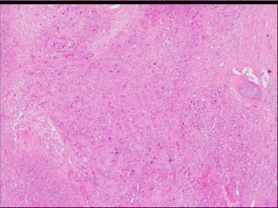
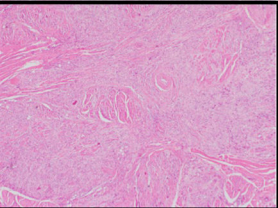
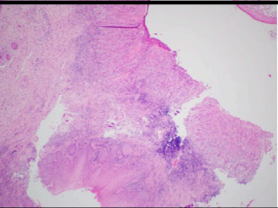
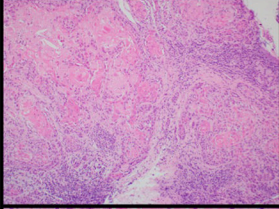
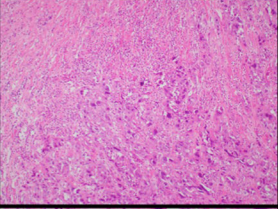
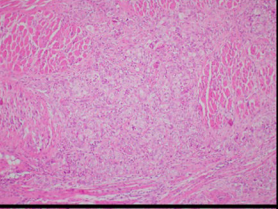
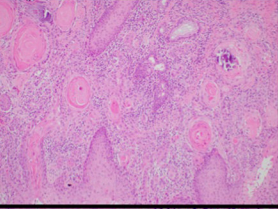
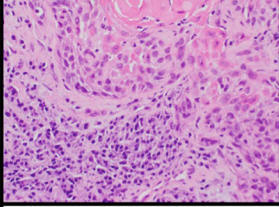
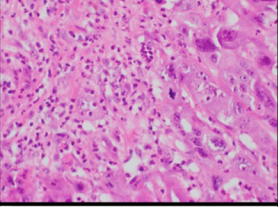
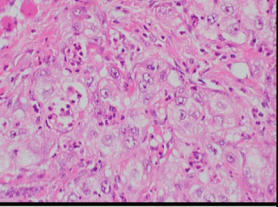
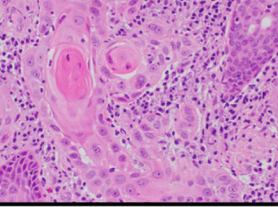
		HN1	HN3	HN4	HN6
Magnification Level	40X				
	100X				
	400X				
Immune Score	Grade	Severe	Moderate	Mild	Moderate
	Lymphoid Infiltrate	Continuous and dense rim (Pattern 1)	Limited or absent (Pattern 3)	Limited or absent (Pattern 3)	Discontinuous dense patches (Pattern 2)
	Neutrophils	0% (0)	90% (3)	80% (3)	5% (1)

Figure 2. Representative formalin-fixed-paraffin embedded tumor sections stained with hematoxylin and eosin (H&E) from cases with an occupational asbestos exposure history and available tumor tissue, with corresponding immune scores.

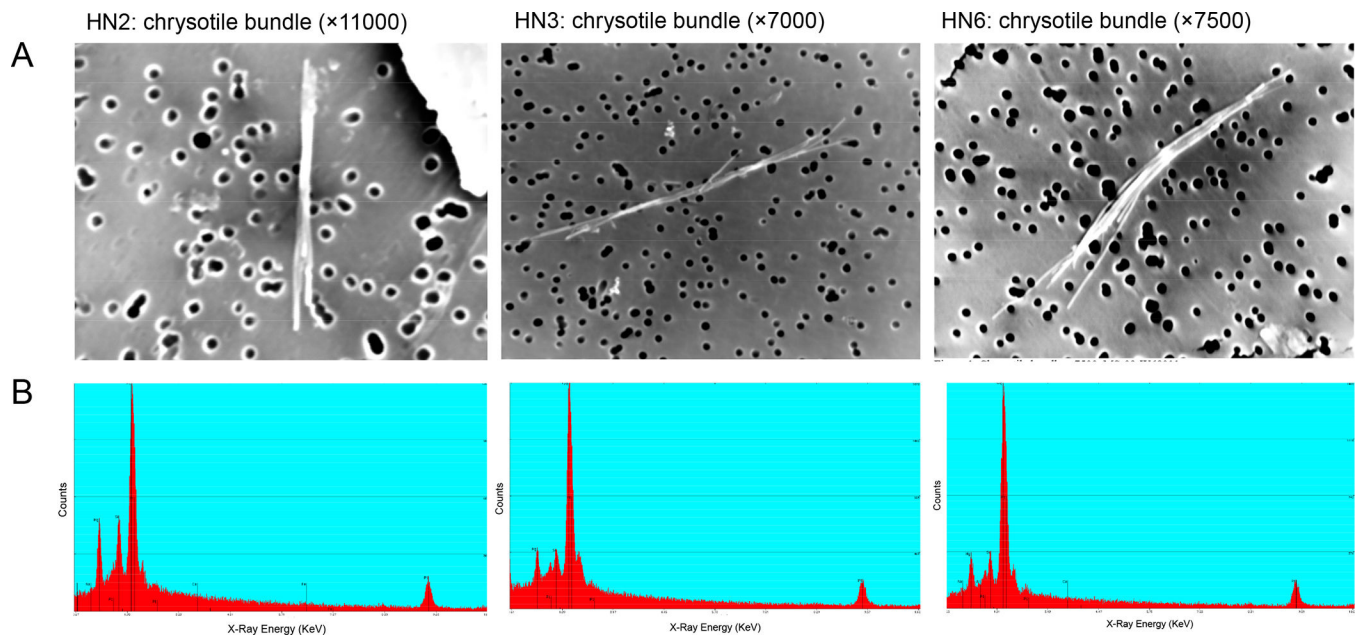


Figure 3. Analysis of tissue mineral fiber content. (A) Scanning electron microscopy (SEM) images of chrysotile asbestos fibers identified in tissue adjacent to laryngeal squamous cell carcinoma tumor in 3 cases with a history of occupational asbestos exposure: HN2, HN3 and HN6. (B) Corresponding energy dispersive x-ray analyzer (EDXA) spectrum consistent with chrysotile asbestos fibers.

Table 1.

Description of study subject characteristics and occupational history.

Case ID	Primary Tumor Site	HPV16 Serostatus	Sex	Age at Diagnosis (years)	Race/Ethnicity	Smoking Pack-Years	Average Alcoholic Drinks per Week	Asbestos-Exposed Occupation(s)/Industry(s)	Years in Asbestos-Exposed Occupation(s)
HN1	Larynx	Negative	Male	61	Caucasian	84.0	7.5	Autobody repair	44.0
HN2	Larynx	Negative	Male	65	Caucasian	0.0	12.0	Space shuttle thermal protection applicator; firefighter	26.5
HN3	Larynx	Negative	Male	62	Caucasian	23.5	99.2	Automotive mechanic	37.5
HN4	Oropharynx	Negative	Male	56	Caucasian	49.5	2.7	Automotive mechanic	5.0
HN5	Oropharynx	Negative	Male	77	Caucasian	5.9	11.8	School custodian/Army artillery	31.5
HN6	Larynx	Negative	Male	66	Caucasian	114.0	19.3	Metal lather	56.0
HN7	Larynx	Negative	Male	64	Caucasian	39.0	80.3	<i>Non-exposed control (truck driver)</i>	0.0

Table 2.

Quantity of adjacent tissue for asbestos fiber count analysis.

Case ID	Number of Cores for Fiber Analysis	Sample Wet Weight ^a (g)
HN1	(1) 2mm core	0.0076
HN2	(1) 2mm core	0.0030
HN3	(3) 2mm core	0.0004
HN4	(4) 2mm core	0.0110
HN5	(1) 2mm core	0.0001
HN6	(3) 2mm core	0.0001
HN7	(5) 2mm core	0.0020

^aMeasured after deparaffinization and rehydration of tissue

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