Professional Oral Care Reduces Carcinogenic Acetaldehyde Levels in Mouth Air of Perioperative Esophageal Cancer Patients: A Prospective Comparative Study

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Acetaldehyde is a potential carcinogen for esophageal cancer, and some oral microorganisms produce acetaldehyde from ethanol or glucose. In this prospective study, we examined the influence of professional oral care on acetaldehyde levels in mouth air of esophageal cancer patients. Acetaldehyde concentrations in mouth air and breath were measured by a portable gas chromatograph, and acetaldehyde production from oral microbiota was also evaluated. Samples were taken from 21 esophageal cancer patients (median age 68 years) and 20 age-matched healthy volunteers (control group) before and after oral care. Postoperative samples were also taken from 17 patients who had undergone surgery. All samples (mouth air, breath, and saliva) were collected 2 to 3 hours after lunch. Oral microbial samples were prepared from saliva. Genotype analysis of alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase-2 (ALDH2) genes revealed no significant differences in the genotypes between the two groups. In the control group, acetaldehyde levels in mouth air showed no significant changes after oral care, while the amount of microbial acetaldehyde production from ethanol was significantly decreased. By contrast, among the patients, acetaldehyde levels in mouth air were significantly decreased after oral care and after operation, while the amount of microbial acetaldehyde production from ethanol showed no significant changes. Moreover, microbial acetaldehyde production from glucose was significantly decreased after operation. Overall, oral health was poorer in the patient group. In conclusion, professional oral care for esophageal cancer patients is effective for reducing acetaldehyde levels in mouth air due to the reduction of microbial count.

Keywords: acetaldehyde; esophageal cancer; genotype; oral care; oral microbiota

Tohoku J. Exp. Med., 2019 September, 249 (1), 75-83. © 2019 Tohoku University Medical Press

Introduction

Esophageal cancer is the seventh-most-common cancer and the sixth-leading-cause of cancer-related death globally (Bray et al. 2018). The overall five-year survival rate is reported to be from 15% to 25%, with propensity for metastasis, and patient prognosis remains poor (Pennathur et al. 2013), making prevention of esophageal cancer crucial. Epidemiologically, heavy drinking and variants of enzymes that metabolize alcohol constitute the major risk factors for esophageal squamous cell carcinoma, which accounts for about 90% of cases of esophageal cancer worldwide (Pennathur et al. 2013; Rustgi and El-Serag 2014; Nieminen and Salaspuro 2018). The incidence of squamous cell carcinoma is highest in Eastern and South-East Asia, followed by sub-Saharan Africa and Central Asia (Arnold et al. 2015). Poor oral hygiene is suggested to be one of the most important risk factors for esophageal squamous cell carcinoma, especially in those high-risk areas (Arnold et al. 2015; Abnet et al. 2018).

Orally ingested ethanol is metabolized to acetaldehyde, mainly by alcohol dehydrogenase 1B (ADH1B) and alcohol dehydrogenase 1C (ADH1C), and acetaldehyde is subsequently metabolized to acetate by aldehyde dehydro-
genase-2 (ALDH2) in the liver (Yukawa et al. 2012). The enzymatic activities of ADH1B and ALDH2 are determined by variants caused by single nucleotide polymorphism (SNP). The reference number of SNP is rs1229984 for ADH1B and rs671 for ALDH2 (Sakiyama et al. 2017). The genetic variant ADH1B*2 increases the ethanol metabolism, causing overproduction of acetaldehyde, because of the amino acid substitution of histidine (His) for arginine (Arg) at position 48. The variant ALDH2*2 is deficient in acetaldehyde catabolism activity, because of the amino acid substitution of lysine (Lys) for glutamate (Glu) at position 504, causing acetaldehyde accumulation (Yokoyama et al. 2006; Li et al. 2009; Yukawa et al. 2012; Sakiyama et al. 2017). A genome-wide association study of esophageal squamous cell carcinoma revealed that the genetic variants ADH1B*2 and ALDH2*2 interact with alcohol consumption to increase esophageal squamous cell carcinoma in Japanese individuals (Tanaka et al. 2010; Yukawa et al. 2012).

Oral health and dental care are reported to be independent risk factors for upper aerodigestive tract tumors, including esophageal cancer (Ahrens et al. 2014). One responsible carcinogen is metabolically-formed acetaldehyde, and not only the host metabolism but also the microbial metabolism contribute to acetaldehyde production (Niimenin and Salaspuro 2018). It is known that Candida and oral bacteria, such as Neisseria and Streptococcus, produce acetaldehyde from ethanol or glucose in vitro (Uittamo et al. 2009; Moritani et al. 2015; Tagaino et al. 2019). Accumulation of microorganisms due to poor oral hygiene might increase ethanol-derived or glucose-derived acetaldehyde production in microbial metabolism (Tagaino et al. 2019). A pilot study with healthy volunteers reported that tongue coating, which serves as a reservoir of oral microflora (Roldan et al. 2003), is associated with acetaldehyde concentration, and suggested that tongue cleaning would be useful for decreasing the acetaldehyde in mouth air (Yokoi et al. 2015). To the best of our knowledge, however, changes of acetaldehyde by oral care before and after operation in esophageal cancer patients have not been reported so far. In this study we estimated the prospective change of acetaldehyde levels in mouth air and acetaldehyde production of oral microbiota originating from saliva in perioperative esophageal cancer patients, and compared them with healthy volunteers. Since the genetic variants ADH1B*2 and ALDH2*2 are risk factors for esophageal squamous cell carcinoma in East-Asian countries (Yukawa et al. 2012), we also examined polymorphism of ADH1B and ALDH2 by genotyping. The aim of this study was to elucidate the possible role of oral care as a preventive strategy for esophageal cancer patients.

Material and Methods

Ethical approval

This study was approved by the Institution Review Board at Tohoku University Graduate School of Dentistry (No. 2015-3-10) and registered in the University hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000020230). The study was performed in accordance with the STROBE guidelines (von Elm et al. 2014). Written informed consent was obtained from all participants.

Subjects

Twenty-one preoperative esophageal cancer patients were registered. Inclusion criteria were as follows: thoracic esophageal cancer; 20 y ≤ age ≤ 80 y; clinical stage I, II, III, according to UICC 7th edition (Rice et al. 2010); ECOG Scale of Performance Status 0, 1. (Oken et al. 1982; Young et al. 2015). Exclusion criteria were as follows: active concomitant malignancy; adults without sufficient ability to judge. Twenty healthy volunteers also participated in the study. Inclusion criteria were as follows: 20 y ≤ age ≤ 80 y; no history of cancer. Exclusion criteria were as follows: antibiotics use in the last month; suffering from respiratory disease, digestive disease, or otolaryngological disorder. The recruitment period was from December 2015 to January 2018. Oral care was performed at the Department of Preventive Dentistry in Tohoku University Hospital.

Sampling procedures

Sampling was conducted for the 21 esophageal cancer inpatients and 20 healthy volunteers before oral examination and oral care and after oral care. Post-operative sampling was also conducted during hospitalization for the 17 esophageal cancer patients who underwent surgery. All samples (mouth air, breath, and saliva) were collected 2 to 3 hours after lunch, to reduce the influence of circadian oscillations of the salivary microbiome (Takayasu et al. 2017).

The order of collecting samples was as follows: mouth air 1 (first time), breath, mouth air 2 (second time), saliva, and buccal swab after rinsing the mouth. Each subject kept their mouth closed for 30 seconds while holding a sterile plastic syringe (10 ml NORM-JECT, Osaka, Japan) for measurement of acetaldehyde. A detailed description of the XG-100 analytical system has been reported previously (Tanda et al. 2014). The XG-100E is a portable gas chromatograph using a SnO2 thick film-type gas sensor. It consists of a semiconductor gas sensor, a separation column, an injection port, a temperature control unit with a heater, a mass flow controller, an air pump, a circuit board with a serial communication interface, a stain-
less-steel air buffer with activated carbon that also functions as an air filter, and a notebook PC with software for data analysis. Air is pumped into the air filter to be cleaned as the carrier gas of the system and the flow rate of the carrier gas is controlled at 20 ml/min by the mass flow controller. The sensor signal is transmitted to the PC at a sampling rate of 0.5 sec and automatically analyzed by the software to determine the concentration of the targeted gas components.

Sampled gases of mouth air and breath in gas bags were injected by syringes and measured immediately after sampling. The mean value of mouth air 1 and 2 was calculated and used as the value of mouth air.

Preparation of microbial samples from saliva

The saliva samples were preserved on ice and brought to the lab immediately after collection. Volumes of saliva samples were recorded. The samples were centrifuged and the supernatants were removed, and the sediments were used as oral microbial samples after washing with saline. The sediments were incubated with 11 mM ethanol and 100 mM glucose in 50 mM potassium phosphate buffer (pH 7) with 0.9% sodium chloride under aerobic and anaerobic conditions. Acetaldehyde produced and released to gaseous phase was measured by a portable gas chromatograph, XG-100E (New Cosmos Electric Co., Ltd., Osaka, Japan). Acetaldehyde in the aerobic condition was measured 2 h after incubation and that in the anaerobic condition 4 h after incubation. The numbers of microorganisms in salivary samples were counted before incubation by an oral bacteria detection apparatus, Bacterial Counter (Panasonic Healthcare, Inc., Tokyo, Japan) (Hirota et al. 2014). The number of microorganisms per unit volume of saliva (1 ml) and acetaldehyde per unit number (1 × 10^7) of microorganisms were calculated.

Oral examination and oral care

Clinical examinations were performed by a single dentist (NT). Subjects received oral examination, hygiene instruction, scaling, professional mechanical cleaning of the teeth surfaces and/or dentures, and tongue cleaning as oral care.

The number of teeth present, use of dentures, the proportion of sites with bleeding on probing (Trombelli et al. 2018) and plaque control record (O'Leary et al. 1972) were examined. Drinking habit was estimated as number of standard drinks (10 g ethanol) consumed by subjects. Smoking history was recorded as the Brinkman index (number of cigarettes smoked per day multiplied by number of years of smoking) (Yoshida et al. 2018).

Statistical analysis

Differences in distributions of categorical and continuous variables between patients and controls were tested by Fisher’s two-sided exact test and by the Mann-Whitney U test, respectively. Distributions of continuous variables at two time points, before and after oral care, within the patient or control group, and before and after operation within the patient group were evaluated via Wilcoxon’s signed-rank test. All P values were two-tailed and those less than 0.05 were considered significant. IBM SPSS Statistics for Macintosh (Version 22.0, IBM Corp., Armonk, NY) was used for statistical analysis.

Results

The clinical stages of the esophageal patients were I (5 cases), II (5 cases), and III (11 cases) according to TNM classification (UICC 7th edition) (Rice et al. 2010). Four of the patients were not able to proceed to operation because of their clinical condition (Fig. 1). The pathological diagnoses were squamous cell carcinoma in 19 patients and carcinosarcoma in 2 patients.

Table 1 lists the baseline characteristics of patients and controls. ADH1B*1/*2 (ADH1B Arg/His genotype) was found in all patients and controls. ALDH2*1/*2 (ALDH2 Glu/Lys genotype) was found in 51% of patients and the rest was ALDH2*1/*1 (ALDH2 Glu/Glu genotype). In controls, ALDH2*1/*2 (ALDH2 Glu/Lys genotype) was 55% and the rest was ALDH2*1/*1 (ALDH2 Glu/Glu genotype). Genotyping of ADH1B and ALDH2 showed no differences between the two groups. However, smoking and alcohol consumption were significantly higher in the patient group. There were no significant differences in the number of teeth

Fig. 1. Flow chart of the present prospective study.
Sampling was conducted in 21 esophageal cancer inpatients and 20 healthy volunteers before oral examination and oral care (T1) and after oral care (T2). Four of the patients were not able to proceed to operation due to their clinical conditions. Postoperative sampling was also conducted in 17 esophageal cancer patients (T3) during their hospitalization.
or use of dentures between the two groups. However, the percentages of plaque control record and bleeding on probing were significantly higher in the patient group. Saliva sediments and number of microorganisms in 1 ml saliva were significantly higher in the patient group than in the control group (Table 1).

Table 2 shows a pre-/post-operative comparison of body weight (BW), body mass index (BMI), albumin (ALB), total lymphocyte count (TLC), white blood cells (WBC), and C-reactive protein (CRP) of the 17 patients who underwent surgery. All of them showed significant differences.

**Change of microbial number per unit volume of saliva after oral care**

Before oral care, the median (interquartile range, IQR) numbers of microorganisms in the control group and patient group were 4.93 (2.21-9.14) × 10^7 cfu/ml saliva and 10.92

| Table 1. Baseline Characteristics. |
|-----------------------------------|
| Patient group | Control group | P value |
| (n = 21) | (n = 20) | |
| Age (years) | 68 (64-74) | 71 (59-76) | 1.000 |
| Sex (male) | 17 (81.0) | 7 (35.0) | 0.004 |
| ADH1B (*1/*2) | 21 (100%) | 20 (100%) | 1.000 |
| ALDH2 (*1/*2) | 12 (51%) | 11 (55%) | 1.000 |
| Drinking | 5 (3-7) | 0 (0-0) | < 0.001 |
| Duration of drinking (years) | 40 (15-47) | 0 (0-0) | < 0.001 |
| Brinkman index | 600 (270-1,000) | 0 (0-0) | < 0.001 |
| Number of teeth | 24 (9-26) | 25 (19-28) | 0.266 |
| Denture wearers | 8 (38.1%) | 8 (40.0%) | 1.000 |
| Plaque control record (%) | 36.2 (26.5-54.0) | 24.6 (17.3-31.0) | 0.007 |
| Bleeding on probing (%) | 4.9 (1.3-30.3) | 1.2 (0.0-3.0) | 0.007 |
| Saliva sediments (mg) | 142.5 (91.0-225.6) | 68.2 (54.6-93.0) | < 0.001 |
| Number of microorganisms (× 10^7 cfu/ml) | 10.92 (3.55-22.47) | 4.93 (2.21-9.14) | 0.045 |

Data are n (%) or median (interquartile range, IQR). Differences in distributions of categorical and continuous variables between groups were tested by Fisher’s two-sided exact test and by Mann-Whitney U test, respectively.

ADH1B, alcohol dehydrogenase; ADH1B*1/*2, ADH1B Arg/His genotype; ALDH2, aldehyde dehydrogenase-2; ALDH2*1/*2, ALDH2 Glu/Lys genotype; Drinking, number of standard drinks (10 g ethanol); Brinkman index, number of cigarettes smoked per day multiplied by the number of years of smoking.

| Table 2. Perioperative information of 17 patients. |
|--------------------------------------------------|
| Preoperation (T2) | Postoperation (T3) | P value |
| BW (kg)       | 61.1 (48.5-63.8) | 55.6 (46.2-61.1) | 0.004 |
| BMI           | 21.7 (20.5-23.4) | 19.9 (18.9-23.0) | 0.004 |
| ALB (g/dL)    | 3.7 (3.5-4.1)     | 3.3 (3.1-3.4)     | 0.002 |
| TLC (× 1,000/µL) | 1.7 (1.5-2.1) | 1.4 (1.2-1.8) | 0.009 |
| WBC (× 1,000/µL) | 5.3 (3.9-7.0) | 7.4 (5.8-9.0) | 0.025 |
| CRP (mg/dL)   | 0.1 (0.0-0.4)     | 0.8 (0.5-2.7)     | 0.046 |

Data are median values (interquartile range, IQR). Differences between two groups were tested by Mann-Whitney U test.

BW, body weight; BMI, body mass index; ALB, albumin; TLC, total lymphocyte count; WBC, white blood cells; CRP, C-reactive protein.
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Acetaldehyde in control group

Acetaldehyde in mouth air and breath samples showed no differences before and after oral care (Fig. 2a). However, the amount of acetaldehyde produced from oral microbial samples incubated with ethanol was significantly decreased after oral care under aerobic ($P = 0.04$) and anaerobic ($P = 0.007$) conditions (Fig. 2b). The amount of acetaldehyde produced from oral microbial samples incubated with glucose was much less than that when incubated with ethanol, it tended to decrease after oral care under aerobic ($P = 0.062$) and anaerobic ($P = 0.075$) conditions (Fig. 2c).

Acetaldehyde in patient group

Acetaldehyde in the mouth air samples from patients decreased significantly after oral care, and after operation. Although acetaldehyde in breath samples was much less than that in mouth air samples, it declined significantly after oral care (Fig. 3a).

In the patient group, acetaldehyde from oral microbial samples incubated with ethanol aerobically and anaerobically showed no significant differences after oral care and after operation. However, acetaldehyde under anaerobic conditions tended to decrease after operation ($P = 0.055$) (Fig. 3b). Acetaldehyde from oral microbial samples incubated with glucose was lower than that with ethanol under aerobic and anaerobic conditions, and showed no significant differences after oral care, but it decreased significantly after operation under aerobic ($P = 0.015$) and anaerobic ($P = 0.015$) conditions.
Discussion

In spite of our expectation that the ratio of variants of \textit{ADH1B} and \textit{ALDH2} would be higher in the patient group, \textit{ADH1B*1/*2} (\textit{ADH1B} Arg/His genotype) was found in all subjects. \textit{ALDH2*1/*2} (\textit{ALDH2} Glu/Lys genotype) was found in 51% of patients and 55% of controls. Both \textit{ADH1B*2} and \textit{ALDH2*2} are prevalent genotypes found in approximately 90% and 50% of the population, respectively, in East Asian countries (Yukawa et al. 2012), and the ratios in our study are concordant with the reported values. The genetic risk factors for esophageal cancer were almost the same between patients and controls, while the environmental risk factors were higher in the patient group due to their amount and frequency of drinking and smoking compared with the control group.

There were no significant differences in use of dentures and number of remaining teeth. However, oral health indicated by plaque control record or bleeding on probing was much poorer in the patient group. Plaque control record is the percentage of tooth surfaces with dental plaque on the gingival margin, and is used as an indicator of oral hygiene status. Bleeding on probing is the percentage of bleeding sites on probing periodontal pockets, and is used as a predictor of periodontal stability (Zimmermann et al. 2015). Higher values of plaque control record and bleeding on probing suggest more oral microorganisms with chronic periodontitis in the patient group. Heavier saliva sediments and greater numbers of microorganisms in saliva suggest poorer oral health of the esophageal cancer patients before oral care (Table 1).

We estimated the correlation coefficients between value of plaque control record and acetaldehyde levels in mouth air before oral care for the patient and control groups. Spearman's rank correlation coefficient was 0.201

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Fig. 3. Changes of acetaldehyde levels in mouth air and breath, and in microbial production after oral care and operation of patient group.

Box plots represent interquartile range, with the middle line representing the median and the whiskers showing the range. Acetaldehyde concentrations before and after oral care (T1 and T2) and before operation and after operation (T2 and T3) were evaluated via Wilcoxon's signed-rank tests.

(a) Acetaldehyde levels in mouth air and in breath.

(b) Amount of microbial acetaldehyde production from ethanol under aerobic condition and anaerobic condition.

(c) Amount of microbial acetaldehyde production from glucose under aerobic condition and anaerobic condition.
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The control group. This suggested a weak positive correlation between the value of plaque control record and mouth acetaldehyde levels in the patient group, and a weak negative correlation in the control group, but the number of subjects was too small to obtain significant results.

Intensive oral care mechanically destroys biofilm and removes oral microbiota, facultative or obligate anaerobes. In the control group, acetaldehyde in mouth air and breath showed no significant difference after oral care. The activity of acetaldehyde production from oral microbiota, however, decreased significantly when incubated with ethanol, and the difference was greater in the anaerobic \( (P = 0.007) \) than in the aerobic condition \( (P = 0.04) \). We interpret this to mean that oral care influenced the composition and metabolic activity of the oral microbiota and reduced the activity of acetaldehyde production from ethanol in aerobic or anaerobic conditions as a whole, so that acetaldehyde production by incubated oral microorganisms decreased. A healthy oral condition achieves homeostasis by regular functional oscillation (Takayasu et al. 2017), i.e., normal eating and swallowing, which allowed the ratio of aerobes or facultative anaerobes as early stabilizers on the tongue or teeth surfaces to recover earlier than anaerobes in the control group. This recovery system of the healthy oral condition might have contributed to the absence of a significant change in acetaldehyde in mouth air after oral care.

In esophageal cancer patients, acetaldehyde in mouth air decreased significantly after oral care. However, the activities of acetaldehyde production of oral microorganisms incubated with ethanol or glucose showed no significant changes. Higher values of plaque control record, bleeding on probing, heavier saliva sediments, and more numerous microorganisms in saliva before intensive oral care indicate poorer oral health including chronic periodontitis with oral microbial dysbiosis (Eloe-Fadrosh and Rasko 2013; Meuric et al. 2017). An imbalance of oral microbial composition and uncontrolled large numbers of microorganisms were expected in the patient group. Since most esophageal patients suffer from impairment of swallowing in addition to a poor oral condition, controlling and maintaining the oral environment may be difficult for them. As the significant difference in microbial numbers between the patient and control groups disappeared after oral care, the decrease of acetaldehyde in mouth air after oral care may have been caused by the reduced number of oral microorganisms involved in acetaldehyde production in the patient group.

After operation, esophageal patients use enteral nutrition without oral feeding until leakage or other problems are confirmed absent by fluoroscopy. We sampled mouth air, breath, and saliva around 14 days (median, IQR: 8–21 days) after operation, according to each patient’s general condition. Acetaldehyde levels in mouth air of patients decreased significantly after operation, while acetaldehyde levels in breath showed no change. Despite enteral nutrition, the surgical stress and absence of oral intake after operation caused decreases in body weight, body mass index, albumin and number of lymphocytes, whereas white blood cells and C-reactive protein as indicators of inflammation were higher than normal values. These postoperative data suggest that surgical stress and a lack of oral feeding may negatively affect maintenance of the oral environment. Consequently, the ratio of some oral microorganisms which metabolize glucose to produce acetaldehyde might decrease after operation.

The patient group showed poorer oral health with more numerous microorganisms in saliva, and difficulty in swallowing. Intensive oral care for patients reduced the acetaldehyde in their mouth air, mainly by decreasing the total number of oral microorganisms, including tongue-coating bacteria, cariogenic bacteria and periodontal pathogens. The periodontal pathogens Fusobacterium nucleatum and Porphyromonas gingivalis have recently been reported in connection with survival of patients with esophageal cancer. Fusobacterium nucleatum was found to be associated with shorter survival of esophageal cancer, possibly by contributing to the acquisition of aggressive tumor behavior through the activation of chemokines (Yamamura et al. 2016). Porphyromonas gingivalis infection is associated with the development of esophageal squamous cell carcinoma and was suggested to be a novel risk factor (Gao et al. 2016). Porphyromonas gingivalis is known to promote the conversion of oral microbiota from symbiotic to dysbiotic by controlling complementary and TLR signaling (Hajishengallis and Lamont 2012; Maekawa et al. 2014). It is possible that the mechanical removal of biofilm by oral care contributes to a decrease of periodontal pathogens such as Fusobacterium nucleatum and Porphyromonas gingivalis.

The analysis of ADH1B and ALDH2 variants can be useful for individualized prevention of esophageal cancer (Tanaka et al. 2010). In our study, however, the genetic risk factor was almost the same between patients and controls. Poorer oral health in addition to histories of heavy smoking and drinking was noticeable in the patient group. Case control studies in Japanese and European populations have suggested that poor oral health is associated with a greater risk of upper aerodigestive tract cancer (Sato et al. 2011; Ahrens et al. 2014). The results of our prospective study are consistent with the previous case control studies, and support the idea that a healthy oral condition inhibits the risk of esophageal cancer by reducing carcinogenic acetaldehyde, even if the genetic risk is high. We thus recommend professional oral care to promote oral health for preventing esophageal cancer.

This study had some limitations. First, we did not investigate the identification of microbiota, although previous studies identified oral microbes based on 16SrRNA gene sequencing (Peters et al. 2017; Belstrom et al. 2018). Second, there was a difference in gender ratio between the patient and control groups because of the open recruitment.
of subjects. Otherwise, there were no significant differences between the two groups in age, number of remaining teeth, denture use, and genetic background of ADH1B and ALDH2. Third, the sampling day after operation depended on the general condition of each patient.

In conclusion, our results suggest that oral care for perioperative esophageal patients is effective for decreasing carcinogenic acetaldehyde in mouth air. This study indicates the positive role of oral care as a preventive strategy for thoracic esophageal cancer even if the genetic risk is high.

Acknowledgments

This study was supported by JSPS KAKENHI Grant Number 15K11408.

Conflict of Interest

The authors declare no conflict of interest.

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