Multidimensional Approach to Understanding Head and Neck Cancer

By

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Abstract

Head and neck is the sixth most common cancer type worldwide, with the highest incidence observed in South and Southeast Asia. Comprised of a heterogeneous group of malignancies, head and neck cancers (HNC) span several anatomical subsites, including the: oral cavity, oropharynx, hypopharynx, nasopharynx, larynx, salivary glands, paranasal sinuses and nasal cavity. While ~90% of these neoplasms are morphologically characterized as squamous cell carcinoma, etiological differences across cancer subsite, along with geographic attributes that influence the variable global incidence and mortality trends, make HNC a therapeutically challenging and behaviorally variable disease.

The goal of this dissertation is to improve the current state of knowledge surrounding HNC through the use of descriptive and molecular epidemiological methods. To do so, the first aim utilized several sources of cancer registry data to assess trends in head and neck, and separately nasopharyngeal, carcinoma to better understand how temporal changes in environmental and behavioral risk factors, as well as advances in clinical practices, influence cancer onset and survival. Culminating in four manuscripts, the first two focused on nasopharyngeal carcinoma (NPC); findings revealed Asian/Pacific Islanders living in the U.S., a non-endemic region, to have comparable rates of NPC incidence as those in endemic Thailand. Interestingly, the Epstein-Barr virus (EBV) related differentiated non-keratinizing subtype was shown to be increasing across nearly all gender and race groups in the U.S., while survival rates appeared to vary.

The remaining studies highlighted the role of decreasing tobacco, alcohol and betel quid use, three major risk factors in HNC development, on decreasing laryngeal and oral cavity cancer rates across the U.S. and Southeast Asia. Nevertheless, as the world's largest tobacco

manufacturer and consumer of ~30% of the world's cigarettes, Chinese males in Shanghai experienced a 9.1% annual increase in laryngeal cancer between 1998 and 2002.

The reductions in tobacco use have elucidated the role of human papilloma virus (HPV) as an important causal factor in oropharyngeal carcinoma (OPC). To date, this etiological shift has almost exclusively been studied and proposed to effect Western, more developed countries. Using descriptive epidemiology in aim one to inform molecular analyses in aim two, the results of these studies challenged previous assumptions. Having undergone rapid socioeconomic change, cancer has superseded infectious disease as the leading cause of mortality in Thailand. In accordance with economic growth, changes in cultural norms have allowed for an etiological transition that has led to an increase in oral HPV infections, and consequently OPC incidence. Although far from the 80% prevalence observed in the U.S., OPC HPV rates in Thailand have increased from 16% in 2012 to 26% in 2017—expecting to exceed 50% by 2030.

Despite medical advances in HNC treatment, 5-year relative survival has remained disappointingly stable at 50-60% over the past few decades. Recent studies have shown the presence of tumor-infiltrating lymphocytes to be an important prognostic tool in guiding HNC treatment. For the last aim, dietary factors, which have previously been shown to be important in HNC risk, progression and prognosis, were assessed and shown to significantly influence tumor immune response and subsequently survival.

Overall, the research presented in this dissertation not only provides a better understanding of HNC on a global scale, but also offers a basis for future studies to aid in elucidating etiology and improving treatment and prevention for this highly disfiguring and deadly disease.

Chapter 1

Introduction

Cancer accounts for one in seven deaths worldwide. When assessing disparity, cancer is the second leading cause of death in high-income countries (after cardiovascular diseases) and the third leading cause of death in low- and middle-income countries (after cardiovascular diseases and infectious and parasitic diseases) [1]. Harboring a complex pathology, incidence and survival rates associated with carcinogenesis are closely linked to social, cultural and socio-economic determinants of health. Although squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer in the world [2], geographic differences, such as the population age structure, prevalence of established risk factors and access to screening and care, all play a key role in making HNSCC both a therapeutically challenging and behaviorally heterogeneous disease.

HNSCC encompasses lesions found in several principal subsites, which include: oral cavity, nasopharynx, oropharynx, and larynx [3]. As with many other cancers, genomic instability is an essential feature of HNSCC [4]; it is fundamental in the transformation of host cells toward malignancy, promotion of invasion and metastasis, and impacts the response of a tumor to treatment. Genomic instability is the most common cause of chromosomal mutation, which is thought to be the most readily observed feature of cancer, and is influenced by the accumulation of DNA damage, lack of proper DNA repair and replication stress. DNA damage response functions to inhibit cell cycle progression through checkpoint signaling for either DNA repair or apoptosis (programmed cell death). In head and neck cancer, the propensity for DNA damage is increased by exposure to a multitude of carcinogens, which likely play a role in explaining the disproportionately high male predominance of this disease.

HNSCC is unique in that each principle subsite is characterized by distinct etiological risk factors and molecular characteristics that inform treatment strategy and prognosis. Nevertheless, despite improvements in HNSCC treatment, 5-year relative survival among afflicted patients remains stagnant at 50-60%. To better understand head and neck cancer on a global scale, this dissertation utilizes descriptive epidemiology methods to inform molecular analyses that can then be translated into the clinical setting in hopes of improving prevention strategies, quality of life, morbidity, and mortality among HNSCC patients.

Lifestyle Factors

The association between HNSCC, tobacco and alcohol use have long been established [5,6]. While these etiological factors may account for the majority of HNSCC cases in the United States, several other behavioral risk factors have been identified elsewhere in the world. One relevant exposure is the use of betel quid, which often consists of areca-nut, slaked lime, betel-leaf and sometimes tobacco. Betel chewing is a common habit in many parts of Asia, and several studies have been published on the risk of betel quid, and its interaction with smoking on head and neck cancer development [7].

In developing countries, an estimated 60% of cancers of the oral cavity, pharynx and esophagus are thought to be associated with micronutrient deficiencies related to a restricted diet low in fruits, vegetables and animal products [8-10]. Several studies have previously investigated the role of individual food groups [11,12] and nutrients [13-16] on HNSCC prognosis. Previous reports have found potential protective effects associated with plasma carotenoids, specifically lutein, α-carotene, β-carotene [13], and lycopene [14] on HNSCC survival. Additionally, a previous study conducted by our group found that pretreatment dietary patterns within a U.S. cohort can be defined into two distinct groupings, and that the group characterized by a diet rich in vegetables, fruit, fish, poultry, and whole grains showed significantly improved survival compared to that characterized by a diet rich in red and processed meats, refined grains and potatoes [17].

Inflammation

Dysregulation of healing processes, chronic infections and immune-mediated diseases have the ability to cause chronic inflammation, which can consequently activate cellular pathways that

constitute risk factors for both cellular transformation and cancer progression[18]. In a chronically inflamed state, cytokines, chemokines, prostaglandins, reactive oxygen and nitrogen radicals accumulate within the affected microenvironment, which if persistent, have the ability to promote prolonged cell survival and to induce cell proliferation [19,20]. The activation of oncogenes and inactivation of tumor suppressor genes in this manner can result in genetic instability and consequently increase the risk of cancer.

The intrinsic cellular circuits associated with cellular proliferation and survival of genetically altered cells can also lead to the production and secretion of inflammatory mediators. This cellular adaptation allows for angiogenesis promotion and aids the cancer in evading the body's protective immune responses [19,21,22]. Several precancerous conditions, namely oral submucosal fibrosis and to a lesser degree, oral lichen planus, are characterized by immune-inflammatory processes that have previously been implicated in carcinogenesis [23]. Although relatively common, a definitive association between these conditions and the development of OSCC has not been established.

Several studies have previously reported increased inflammation associated with both alcohol consumption and western dietary patterns [18]. Hyperadiposity has also previously been linked to chronic subclinical inflammation through a number of mechanisms including: switching the phenotype of adipose-tissue macrophages from the M2 anti-inflammatory phenotype to M1 proinflammatory phenotype, increased production of proinflammatory cytokines (eg. TNF, interleukins IL-6 and IL-1β), and elevation in glucose, free fatty acids, leptin, insulin and insulin-like growth factor (IGF1) [24,25]. High consumption of red meat has also been implicated in carcinogenesis though proposed mechanisms such as increased insulin resistance, oxidative stress and inflammation [26-28]. Whole grains, on the other hand, have been shown to be protective against tumorigenic processes by improving insulin sensitivity, lowering insulin and glucose levels, and by inhibiting inflammation and oxidative stress [28,29]. Although consensus on dietary studies largely remain inconclusive, there is reason to believe that diet/dietary effects impact cancer risk independently and through inflammatory pathways.

Infectious Agents

Human papillomavirus (HPV)

Human papillomavirus (HPV) has been associated with oral squamous cell carcinoma (OSCC) for over thirty years [30]. Nevertheless, recent cultural shifts leading to decreased tobacco use and increased HPV prevalence have allowed for an epidemiological change that has highlighted the role of HPV in HNSCC pathology. This subset of the disease is seen more commonly in younger patients, is associated with an improved prognosis and consequently has a different treatment profile from non-HPV oropharyngeal HNSCC [31-34]. In the western world, most of these infections are attributed to "high risk" HPV subtypes 16 and 18 [35], and have been shown to be present in up to 60-80% of non-oral HNSCC and ~30% of OSCC [36-39]. On the molecular level, HPV associated HNSCC tumors often present with elevated p16 levels, and an absence of p53 and RB1 mutations [40,41]. These characteristics are unique to HPV positive HNSCC, as HPV negative tumors characteristically have inactivated p53 and p16 tumor suppressors and amplified RB1, consequently promoting cell cycle progression [42]. Nevertheless, very little is known about HPV as a potential etiological factor elsewhere in the world.

Epstein-Barr Virus (EBV)

Epstein-Barr virus (EBV) belongs to the Herpesviridae family, which consists of eight viruses grouped into three subcategories: Alpha, Beta, and Gamma. In addition to acute disease, one of the hallmarks for these viruses is their ability to maintain latency; the subgroupings allow for differentiation of these viruses based on factors such as reproductive cycle, the particular cells which harbor the latency, and the consequent effect of potential re-activation later in life. EBV is one of the two gamma herpes viruses (HHV-8 being the second), characterized as such based on their ability to replicate in lymphoblastoid cells. Unlike HHV-8, EBV maintains latency in the lymphoid tissue, allowing areas of the oral cavity, such as tonsils and adenoids, to be primary regions of entry and egress of the virus [43]. EBV initially infects epithelial cells in the oropharynx, where it replicates and consequently is able to infect B-cells by binding to the CD21 receptor in the B-cell surface, resulting in the internalization of the virus [44]. Once infected, the cell can either proceed into the lytic phase (leading to new virions) or a latent phase (allowing the cell to transform to have nearly unlimited growth potential). Reactivated EBV infected cells have been shown to have the ability to transform into various types of malignancies, including: Burkitt

lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma and gastric carcinoma [43]. Approximately 95% of the world's population harbors an asymptomatic, life-long infection with EBV, nevertheless, only a small proportion presents with an EBV-related cancer [45]. Focusing on head and neck cancer, nasopharyngeal carcinoma has been sub-characterized into three histological groupings by the World Health Organization (WHO): keratinizing squamous cell carcinoma (type I) and non-keratinizing squamous cell carcinoma (further divided into differentiated and undifferentiated) and basaloid squamous cell carcinoma (WHO type 3) [46,47]. While basaloid squamous cell carcinoma is incredibly rare, both classes of non-keratinizing SCC are associated with EBV infection, cumulatively accounting for an estimated 80% of worldwide nasopharyngeal cancers [47,48]. According to GLOBOCAN 2012, 71% of all new nasopharyngeal cancer cases were reported in east and southeast Asia [49]. EBV is commonly classified into two groups: type 1 (or A) and type 2 (or B), and diverge through their genomic differences within a subset of latent genes that encode the EBV nuclear antigens [50,51]. Although little is currently known about the prevalence of EBV in head and neck cancer in Thailand, one study investigating the role of EBV in nasopharyngeal cancer found high incidence of type-1 EBV in cases and controls (96.0% and 97.7%, respectably).

Tumor Infiltrating Lymphocytes

Recent cancer studies suggest tumorigenesis to be an immunologic disease as well as a genetic one. Immune cell infiltration (TILs) plays a significant role in HNSCC due to the close proximity of these lesions to the Waldeyer's ring; this allows for dense infiltration of immune cells into the tumor, which has been shown to be prognostically significant in guiding treatment decisions [52]. Additionally, HPV associated oropharyngeal squamous cell carcinomas have been reported to have deep lymphocytic infiltrates and are thought to evolve with immune cells specific for viral antigens [53]. Although HPV positive HNSCCs commonly display nodal metastasis, the overall and progression-free survival rates are usually better than HPV negative HNSCC. One hypothesis for this phenomenon is that the body's immune response against HPV may aid in the destruction of the tumor [54,55].

Chemotherapy and radiotherapy are two of the primary treatment options for HNSCC patients. In addition to these classic techniques, anti-tumor immune activation has been repeatedly noted as

an important mechanism in maximizing treatment efficacy [56]. In regards to chemoradiotherapy, increased levels of CD3 and CD8 T-cell infiltration have been associated with improved response to treatment [57]. That being said, recent literature highlighted the importance of the infiltration pattern of these immune cells, noting the importance of their presence within or in close proximity to the tumor cells, rather than in the tumor stroma or the periphery, on prognostic value [58].

Despite advances in both our knowledge of HNSCC biology and treatment, head and neck cancer remains a highly disfiguring disease with a high mortality rate. With an increase in the world's aging population and increasing incidence of chronic diseases in developing countries, more research is needed to address global risk factors leading to this disease.

Specific Aims

This dissertation aims to advance our understanding of the heterogeneity that exists in the distribution, etiology, characterization and survival of head and neck cancer on a global scale. **Figure I.1** illustrates the conceptual framework for these aims. Using both descriptive and molecular epidemiology methods, the following aims address critical gaps in the literature relevant to cancer monitoring, prevention and treatment.

Specific Aim 1: Characterize and compare age-standardized incidence and mortality rates of head and neck cancer by sub-site and gender across, and within, South Asia, Southeast Asia and the United States.

Hypothesis 1: Due to cultural, geographic and policy-driven variability in the proposed geographic locations, variations in risk and thereby prevalence are expected to be observed. Head and neck cancer is a male dominant disease, which is not expected to drastically change between settings. Site-specific analyses may reflect dissimilarity, largely due to frequency in smoking patterns, betel quid use, as well as HPV and EBV prevalence.

Specific Aim 2: Use molecular epidemiology methods to assess HPV prevalence in oropharyngeal carcinoma in Thailand, assess utility of p16 staining as a surrogate measure of

HPV in this setting, and compare patient characteristics and tumor presentation to those in Western countries.

Hypothesis 2: Based on evidence derived from descriptive epidemiology studies that suggest increasing oropharyngeal carcinoma incidence in Thailand, it is reasonable to believe that the etiological shift to HPV+ cancer has similarly begun to occur in Southeast Asia.
Specific Aim 3: Investigate the potential role of pretreatment diet on tumor infiltrating lymphocytes by assessing both individual nutrients and dietary patterns within the UM-SPORE cohort.

Hypothesis 3*:* Previous literature has shown that foods typically associated with a western diet may impact immune response by increasing inflammatory reactions, impacting immunosurveillance and influencing factors related to cellular proliferation (eg. insulin, leptin, etc.). Consequently, it is reasonable to believe that both individual dietary nutrients as well as dietary patterns may impact TILs, with increasing TIL counts being associated with higher nutrient consumption and a 'whole foods' rather than 'western' diet.

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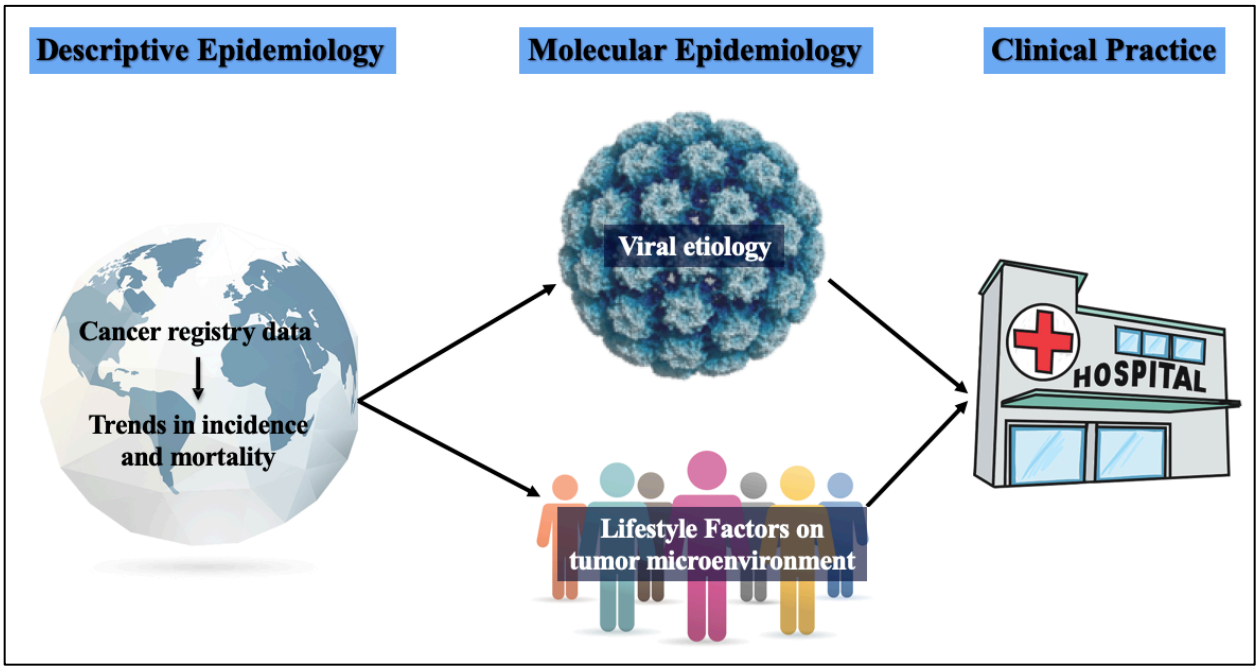
Figure 1.1 Conceptual Framework



Chapter 2

Subtype Specific Nasopharyngeal Carcinoma Incidence and Survival Trends: Differences Between Endemic and Non-endemic Populations

Abstract

Background: While nasopharyngeal carcinoma (NPC) is rare in non-endemic regions such as the North America, endemic countries, such as Thailand, continue to struggle with high incidence and mortality burden. NPC has a complex etiology that varies by histological subtype. As Thailand undergoes rapid socioeconomic development, it is important to assess temporal trends in subtype specific NPC.

Methods: NPC cases from 1990-2014 were identified using the International Classification of Diseases for Oncology (ICD-O) code C11 from the Chiang Mai, Khon Kaen, Lampang, and Songkhla cancer registries and compared to Asian/Pacific Islanders (A/PI) from the US SEER program. Age-standardized incidence rates and changes in annual percent change (APC) for overall and subtype specific NPC were assessed using R and Joinpoint regression software. Kaplan Meier curves were generated in SAS to evaluate differences in survival by sex, year of diagnosis and histological subtype. Five-year relative survival estimates were calculated between 2000-2014.

Results: Non-keratinizing NPC predominated across all registries except Songkhla, where the keretinizing subtype made up ~60% of all reported cases. Incidence of keratinizing NPC significantly decreased among Chiang Mai males between 1996 and 2014 (APC: -13.0 [95%CI: -16.2, -9.6]), Songkhla females (APC: -4.0 [95%CI: -7.4, -0.5]) and males between 2006 and 2014 (APC: -15.5 [95%CI: -25.0, -4.7]), as well as A/PI females (APC: -5.1 [95%CI: -6,7, -3.4])

and males (APC: -4.8 [95%CI: -5.9, -3.7]). Non-keratinizing NPC increased among Songkhla males (APC: 4.3 [95%CI: 1.8, 6.9]). The keratinizing subtype exhibited the worst survival, while the non-keratinizing undifferentiated subtype had the best survival. Although US A/PI had the highest 5-year relative survival estimates, among the Thai registries Chiang Mai had the best and Lampang the worst survival.

Conclusion: Although US A/PIs exhibited similar rates of NPC as seen in the endemic Thai population, improved tobacco control has led to a decrease in keratinizing NPC incidence irrespective of geography. Additionally, while challenges associated with access to care may still exist among rural Thais, chemoradiation was shown to confer a survival benefit in non-keratinizing NPC treatment.

Introduction

Arising in the epithelial lining of the nasopharynx, nasopharyngeal carcinoma (NPC) is uniquely characterized by its striking racial and geographic variation. In most of the world, including the United Stated, NPC is considered to be a rare malignancy, with age-adjusted incidence among both genders reported to be less than one per 100,000 population [1]. Nevertheless, in endemic regions such as southern China, Southeast Asia, the Middle East and North Africa, NPC continues to contribute to a large proportion of the overall cancer burden [2]. The World Health Organization (WHO) classified NPC into three distinct histological subtypes: keratinizing squamous cell carcinoma (WHO type 1), non-keratinizing carcinoma (WHO type 2), which can further be divided into differentiated and undifferentiated, and basaloid squamous cell carcinoma (WHO type 3) [3,4]. While basaloid squamous cell carcinoma is very rare and sparsely mentioned in the literature, in high-incidence countries, non-keratinizing squamous cell carcinoma is considered to be the predominant histological subtype, accounting for an estimated 95% of cases [2,5,6]. Interestingly, keratinizing squamous cell carcinoma comprises the vast majority of NPCs in non-endemic regions, believed to be associated with alcohol and tobacco consumption patterns [7].

Several epidemiological studies have established the WHO histological classifications as independent prognostic factors for NPC survival [8,9]; non-keratinizing carcinoma has been shown to have significant survival advantages when compared to keratinizing squamous cell carcinoma, with 5-year survival rates of 51% and 6%, respectively [10]. Epstein-Barr virus (EBV) has long been implicated as a causal factor in NPC, as well as several other malignancies [11] and is primarily associated with non-keratinizing carcinoma [12,13]. Despite being ubiquitous, infecting and persisting latently in over 90% of the world's population [14], and with numerous studies finding associations between anti-EBV antibodies, tumor burden and prognosis [15-29], there has been no strong evidence to suggest a correlation between the distribution of EBV strains and international patterns of NPC [2].

Located in Southeast Asia, Thailand is an endemic nation for NPC. Rapid socioeconomic development in this region during the past few decades has led to a decrease in communicable diseases and an increase in cancer related mortality [30]. Using data from four of Thailand's 16

province- and regional- based registries [31], we compared age standardized NPC incidence and mortality rates across Thailand to those in the Asian/Pacific Islander (A/PI) population within the United States, paying particular attention to variability in histological classifications across these regions.

Methods

Data Selection and Criteria

Cancer incidence data between 1990 and 2014 were obtained directly from the Chiang Mai, Khon Kaen, Lampang and Songkhla cancer registries in Thailand [32-35] as well as the Surveillance, Epidemiology, and End Results Program 9 (SEER) [36]. For each Thai registry, population estimates by year, age and sex were based on decennial census data from 1990, 2000, and 2010, which were conducted by the Thai National Statistical Office [37,38]. Annual intercensal population estimates for the various provinces were calculated using a log-linear function by 5-year sex-specific age groups. Population counts beyond 2010 were modeled by the Office of the National Economic and Social Development Board [39,40].

Nasopharyngeal cancer cases were identified in the various registries using the International Classification of Diseases for Oncology (ICD-O) code 'C11' and assessed as sex stratified, temporal trends in incidence rates aggregated over 5-year age groups (0-85+). All malignant nasopharyngeal cancer cases identified between the years of 1990 and 2014 were used for these analyses. In addition to cancer site, case information included age, sex, date of diagnosis, stage, histology, morphology, vital status and date last seen. Histological groupings were created according to criteria specified by the WHO International Classification of Diseases for Oncology (ICD-O-3) [3,41] and included: keratinizing squamous cell carcinoma (ICD-O histology codes 8070 and 8071), differentiated non-keratinizing carcinoma (ICD-O histology codes 8072 and 8073), undifferentiated non-keratinizing carcinoma (ICD-O histology codes 8020, 8021, and 8082) and 'carcinoma not otherwise specified' (ICD-O histology code 8010).

Incidence trends

Incidence trend analyses were conducted using Joinpoint Regression Program version 4.5.0.1. [42] to assess trends under a log-linear model and to compute the annual percent change (APC) in age-standardized incidence rates. The aforementioned program utilizes a Monte Carlo permutation method in order to assess number of joinpoints, slope in the trends, as well as the corresponding significance [43]. Sex stratified trends were assessed by registry and morphological subtype. In circumstances where no cases were reported within a given year, a half-case was added to the age strata with the largest population to enable computation on the log-linear scale [32,43,44]. In order to improve comparability between the various Thai registries and SEER, Segi (1960) standardization was applied [45,46]. R-statistical software version 3.3.3 [47] was used to shape the data and generate graphs based on Joinpoint outputs. Due to small sample sizes of sex stratified differentiated and undifferentiated non-keratinizing carcinomas within the Thailand registries, incidence trends for these morphological subtypes were grouped into a single non-keratinizing category. The A/PI race group was selected as a comparison group from SEER.

Survival Analysis

Kaplan Meier curves assessing differences in survival by sex, year of diagnosis (in five-year groupings) and histological subtype were generated for each registry and strata were compared using log-rank tests. Since Thailand does not have data on expected survival by age, sex, calendar year and province, a life table was ascertained from the WHO Global Health Observatory data repository for the years of 2000-2014 to calculate 5-year relative survival among the Thai registries. Five-year relative survival was computed among Asian/Pacific islanders, for the corresponding years, by sex and subtype, using the Kaplan-Meier method in SEER*Stat 8.3.5.

Results:

Between 1990 and 2014, 1,894 cases of NPC were diagnosed among A/PIs in the United States, 934 in Chiang Mai, 704 in Khon Kaen, 549 in Songkhla and 390 in Lampang. Cases were found

to be predominantly male across all the registries, with mean age at diagnosis ranging from 47-53 years. Among A/PI in the US, the non-keratinizing subtype predominated, making up 51% of cases for both males and females. In Chiang Mai, Khon Kaen and Lampang, non-keratinizing nasopharyngeal carcinoma constituted 66-79% of cases, with no significant differences in distribution between genders within each given registry. Interestingly, in Songkhla the majority of cases were found to be of the keratinizing subtype, comprising about 60% of all reported NPCs (**Table 2.1**).

At the beginning of the study period, in 1990, US A/PIs had the highest overall NPC incidence, with an expected age standardized rate (EASR) of 4.48 and 1.60 per 100,000 among males and females, respectively. As time progressed, incidence among A/PI men decreased with an APC of -1.9 (95%CI: -2.6, -1.2) and -1.8 (95%CI: -2.8, -0.8) among women. Declines in NPC incidence were similarly observed among Chiang Mai males, at an APC of -2.2 (95%CI: -3.4, -0.9). Khon Kaen and Lampang presented with suggested increases in nasopharyngeal cancer incidence among both sexes, largely driven by increases in the non-keratinizing subtype. Accounting for these changes in trends, in 2014, US A/PI males remained the highest incident group of NPC (EASR: 2.81 per 100,000), while Lampang emerged as having the highest rates among women (EASR: 1.28 per 100,000) (**Figure 2.1**).

Assessing trends by the presence of keratinization revealed consistent decreases in the keratinizing NPC sub-type; significant decreases were observed among Chiang Mai men between 1996 and 2014 (APC: -13.0 [95%CI: -16.2, -9.6]), Songkhla females (APC: -4.0 [95%CI: -7.4, -0.5]) and males between 2006 and 2014 (APC: -15.5 [95%CI: -25.0, -4.7]), as well as A/PI females (APC: -5.1 [95%CI: -6,7, -3.4]) and males (APC: -4.8 [95%CI: -5.9, -3.7]). Conversely, non-keratinizing NPC appeared to be increasing, particularly among men, across all the registries except Chiang Mai and the US. This observation was found to have the greatest significant impact in Songkhla males, with an APC of 4.3 (95%CI: 1.8, 6.9) (**Figure 2.1**).

Sex stratified Kaplan-Meier (KM) plots demonstrated significantly improved survival among females in Songkhla, Khon Kaen and Chiang Mai (logrank p: 0.01, 0.002 and 0.001, respectively) but no differences across the other registries.

The keratinizing squamous cell carcinoma subtype was found to elicit the worst survival, with significant differences noted in Chiang Mai (logrank p: <0.0001), Songkhla (logrank p: <0.0001) and US A/PIs (logrank p: 0.0004). While the nonkeratinizing differentiated subtype appeared to provide the greatest survival advantage in Songkhla, undifferentiated NPC was associated with improved survival among A/PIs. Khon Kaen was the only registry to demonstrate improved survival with later years of diagnosis (logrank p: 0.002) (**Figure 2.2**). Five-year relative survival estimates, between the years of 2000 and 2014, closely mirrored the KM results. Although US A/PI had the highest 5-year relative survival estimates, Chiang Mai had the best 5-year relative survival among the Thai registries, while Lampang had the worst survival (**Table 2.2**).

Discussion

While numerous studies on nasopharyngeal carcinoma have been conducted within Chinese populations [48-52], this is the first paper, to our knowledge, to assess temporal trends in NPC incidence and mortality across Thailand in comparison to Asian/Pacific Islanders in the United States. As an endemic country for NPC undergoing rapid economic development, it is important to evaluate the role of both preventative risk factors on incidence, as well as changes in healthcare and treatment protocols on survival among NPC patients in Thailand. In this study, we found overall NPC incidence to be decreasing among US A/PIs of both sexes, as well as Chiang Mai males; nevertheless, the remainder of our study sites displayed stagnant NPC incidence. We additionally found distinct survival differences by sex, registry, and subtype, reflective of the variability of population dynamics, cultural norms and access to care.

Use of combustible tobacco has repeatedly been shown to influence the risk of cancer development across a number of head and neck cancer sites, including the nasopharynx. A recent study in Singaporeans found that current smokers exhibited a four-fold increase in NPC development when compared to never smokers, while ever-smokers exhibited a 2-fold increase [53]. Similar findings have been reported in Thailand [54,55], China [56,57], Taiwan [58], the Philippines [59] and the United States [7,60-62]. A meta-analysis of the current literature demonstrated that the risks of combustible tobacco use on NPC were particularly relevant to keratinizing squamous cell carcinoma [53]. Largely due to cultural norms, male smoking rates are much higher than those

among females across Thailand; nevertheless, Thailand does display geographic variability in smoking, with the south having the highest smoking prevalence [63]. Initiated in 1991, Thailand was among the first countries in Asia to implement strict tobacco control policies, including bans on advertisements and smoking in certain public places, health warnings and taxation [64-67]. According to the Tobacco Control Research and Knowledge Center, smoking prevalence has decreased across Thailand, but smoking behaviors remain highest in the south (decreasing from 60.9% in 1994, to 49.9% in 2007) [68,69]. The greater proportion of male smokers likely contributes to the higher incidence of keratinizing squamous cell carcinoma among males observed across all study sites. Songkhla (located in southern Thailand) was the only registry to exhibit higher keratinizing rather than non-keratinizing NPC incidence, likely due to the higher smoking rates within the region.

Keratinizing squamous cell carcinoma typically predominates in non-endemic regions such as the United States [70]. Nevertheless, previous studies have shown that Asian Americans continue to exhibit higher rates of NPC, particularly the non-keratinizing subtype, when compared to other race groups [70,71]. In this study, we found US A/PIs to have the highest age standardized rate of NPC, when compared to the Thai registries. Decreases in incidence over the time period resulted in Lampang females superseding US A/PI females, US A/PI males remained the highest incident group. Although, this study validated previous observations of a predominance of non-keratinizing NPC among this population, the decreased incidence observed within overall NPC is largely attributable to decreases in keratinizing squamous cell carcinoma, while the non-keratinizing subtype remained stagnant.

While non-keratinizing NPC is believed to present with a more aggressive behavior and a higher risk of distant metastasis, keratinizing squamous cell carcinoma has been reported to be less responsive to treatment [72]. Historically, radiotherapy has been the cornerstone of treatment across NPC subtypes. In the minority of patients diagnosed in early stages, NPC has relatively high sensitivity to radiation, but this treatment has been shown to fail among patients with locally advanced disease [72]. More recently, concurrent chemo-radiation treatment has been shown to improve survival among non-keratinizing locally advanced patients, particularly those with undifferentiated tumors [72,73]. Chiang Mai was the first registry location in Thailand to implement

the use of chemoradiation in NPC treatment, beginning enrollment in a clinical trial in 1989 and adapting the treatment into practice in 1996 [74]. Shortly upon completion of the phase 3 randomized Intergroup 0099 Study in 1995 [75], the US adopted chemoradiation as the primary treatment for advanced stage NPC; Khon Kaen, Lampang and Songkhla similarly implemented the new treatment guidelines soon after the US [76,77]. Coinciding with the literature, our study found significantly worse survival among those diagnosed with keratinizing squamous cell carcinoma in Chiang Mai, Songkhla, and US A/PI. Undifferentiated non-keratinizing NPC appeared to be associated with improved survival in Chiang Mai, Lampang and the US, likely due in part to tumor treatment response, and in part to the earlier age of onset for this cancer subtype (data not shown) [78]. Five-year relative survival results indicated that US A/PIs have the best survival, regardless of cancer subtype. Among the Thai registries, Chiang Mai proved to provide the best survival rates, likely due to the early adaptation of chemoradiation.

Although the early adaptation of improved treatment modalities may improve patient survival, equal access to care remains a problem in parts of Thailand. Northern Thailand is unique in that it encapsulates the 'hill tribe' population. These individuals reside in the mountain slopes and are divided into six primary groups: Akha, Lahu, Hmong, Lisu, Yao and Karen. In addition to having different cultural practices, the hill tribes have unique languages, as well as beliefs that influence their health care practices [79,80]. Originating largely from Southern China (a high incidence region for NPC), the Akha are the largest tribe, comprised of an estimated 70,000 individuals [80]. Historically migrant populations, many hill tribe residents did not have legal identification and were not considered Thai citizens until recently. A 2014 report by the Mae Fah Luang District Office estimated that 89% of hill tribe residents had access to health care under the Thai Universal Coverage System, which was introduced in 2000 [81,82]. In 2011, the National Health Examination Survey Office reported that smoking rates among Ahka young adults was higher than that of Thai young adults (28% and 19.9%, respectively) [83]. While exhibiting various high risk behaviors, previous studies have demonstrated that factors such as language barrier/illiteracy rates, lack of transportation, financial restrictions and reliance on traditional medical practices are all factors that contribute to health disparities within hill tribe populations [80,82,84,85]. These various obstacles consequently impact utilization of both treatment and preventative care. Unlike trends observed in Khon Kaen and Songkhla, the Chiang Mai and

Lampang registries appear to have worsening survival with successive years of diagnosis, particularly corresponding to the initiation of universal healthcare. Due to limitations in access to care, these trends may be attributable to an increase of hill tribe patients, who would likely be diagnosed at later stages and be less willing and able to comply with modern treatment protocols.

Differences in data collection methods and population composition pose challenges to comparability between registries. Although Thailand's cancer registries have been verified to have high quality data [44,86], data collection methods vary from passive, to active, to a combination of the two [87-90]. Additionally, while population-based cancer registries allow results to be generalized to nearby provinces, it is difficult to quantify the precise number of unreported cancer cases, particularly within rural areas. Finally, changes to histology practices, such as the introduction of immunohistochemistry staining, may influence subsite specific classification, leading to misclassification bias. This effect is likely observed in the Chiang Mai and Songkhla registries, where the apparent trends between keratinizing and non-keratinizing NPC incidence invert around 1996 and 2006, respectively.

In conclusion, albeit not being an endemic country for NPC, US A/PIs appear to have comparable incidence rates to those observed in the endemic Thai population—even exceeding the cancer burden observed among Thai males. This study also demonstrated the role of tobacco control on keratinizing NPC incidence, as well as the survival benefit associated with the introduction of chemoradiation in non-keratinizing NPC treatment. Finally, it puts into perspective the challenges associated with access to care in rural populations, irrespective of the introduction of universal health care. In order to address this and other potential health disparities in these communities, local health professionals should be encouraged to develop culturally appropriate health promotion and educational programs in order to aid in curbing high-risk behaviors and improving access and acceptance of care.

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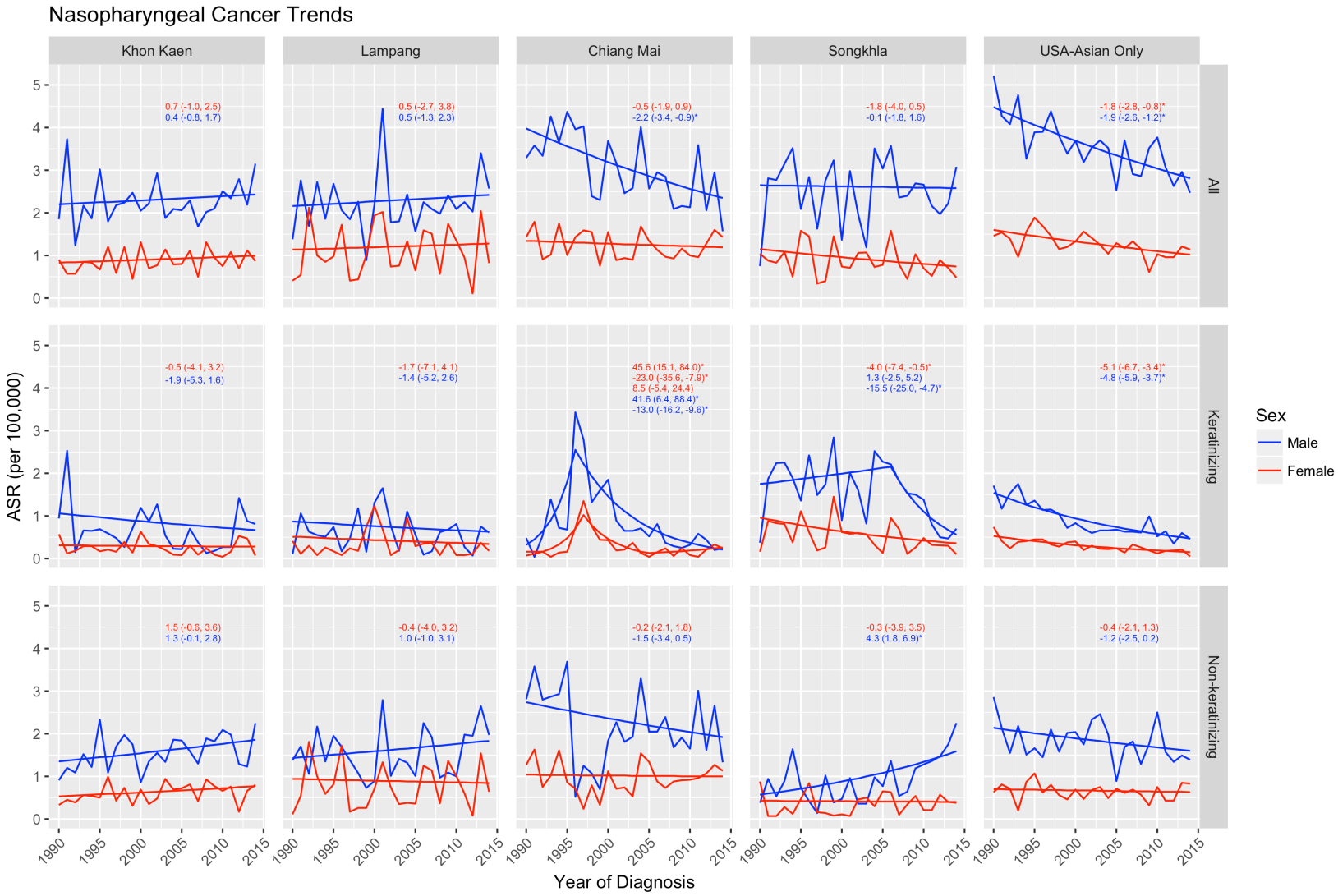
Table 2.1: Distribution of Cases by Gender and Registry (1990-2014)

	USA-Asian/Pacific Islander		Chiang Mai		Khon Kaen		Songkhla		Lampang	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean age (SD), y	52.87 (13.83)	51.76 (15.31)	52.76 (14.11)	51.97 (14.21)	53.02 (14.78)	51.50 (14.46)	51.08 (14.53)	47.17 (16.29)	53.26 (14.62)	51.97 (16.75)
Number of Cases, N (%)	1337 (70.59)	557 (29.41)	650 (69.59)	284 (30.41)	491 (69.74)	213 (30.26)	399 (72.68)	150 (27.32)	261 (66.92)	129 (33.08)
Cell Type, N (%)										
Keratinizing	320 (23.93)	122 (21.90)	178 (27.38)	59 (20.77)	139 (28.31)	57 (26.76)	240 (60.15)	86 (57.33)	72 (27.59)	37 (28.68)
Non-Keratinizing	686 (51.31)	284 (50.99)	466 (71.69)	223 (78.52)	344 (70.06)	150 (70.42)	156 (39.10)	60 (40.00)	178 (68.20)	85 (65.89)
Differentiated	284 (41.40)	130 (45.77)	231 (49.57)	81 (36.32)	199 (57.85)	89 (59.33)	37 (23.72)	14 (23.33)	101 (56.74)	47 (55.29)
Undifferentiated	402 (58.60)	154 (54.23)	235 (50.43)	142 (63.68)	145 (42.15)	61 (40.67)	119 (76.28)	46 (76.67)	77 (43.26)	38 (44.71)
NOS	331 (24.76)	151 (27.11)	6 (0.92)	2 (0.70)	8 (1.63)	6 (2.82)	3 (0.75)	4 (2.67)	11 (4.21)	7 (5.43)
NOS=not otherwise specified										

Table 2.2: 5-year Relative Survival by Registry, Sex and Histology 2000-2014

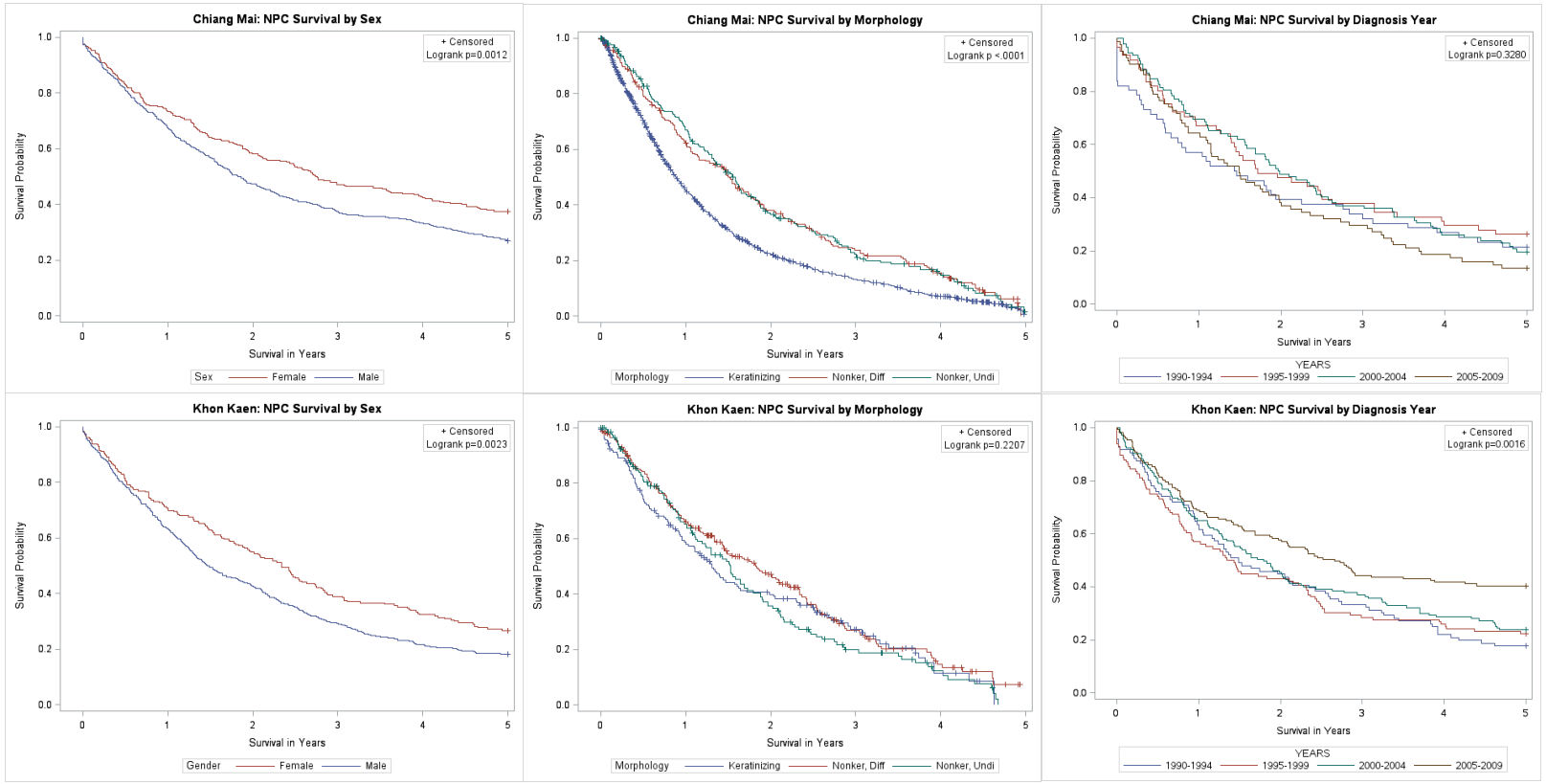
	USA- Asian/Pacific Islander		Chiang Mai		Khon Kaen		Songkhla		Lampang	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number of Cases, N (%)	824	338	395	188	341	151	270	97	181	90
Cell Type, N (%)										
Overall Nasopharyngeal	64.22 (60.46, 67.72)	69.14 (63.27, 74.27)	29.75 (19.84, 39.67)	39.90 (22.05, 57.74)	20.59 (10.89, 30.29)	18.80 (10.76, 26.84)	23.94 (13.99, 33.88)	27.32 (15.08, 39.57)	15.74 (2.41, 29.07)	15.91 (0.00, 32.10)
Keratinizing	54.28 (45.44, 62.28)	52.72 (38.28, 65.26)	29.67 (11.66, 47.67)	36.02 (9.28, 62.76)	13.12 (1.57, 24.67)	25.89 (14.93, 36.85)	31.87 (17.23, 46.51)	33.20 (17.54, 48.86)	18.24 (0.00, 40.73)	13.76 (0.00, 27.58)
Non-Keratinizing	70.79 (65.83, 75.16)	73.54 (65.57, 79.95)	30.76 (20.74, 40.79)	40.46 (27.51, 53.41)	22.54 (15.04, 30.05)	20.88 (11.64, 30.12)	21.69 (10.85, 32.53)	27.87 (10.27, 45.46)	9.70 (2.03, 17.36)	15.48 (1.61, 29.34)
Differentiated	69.37 (61.32, 76.08)	70.53 (58.11, 79.88)	27.35 (11.52, 43.18)	43.95 (23.17, 64.83)	19.42 (8.03, 30.81)	20.82 (7.38, 34.27)	17.40 (0.00, 37.59)	3.36 (0.00, 12.01)	9.79 (0.47, 19.12)	9.05 (0.00, 25.94)
Undifferentiated	71.66 (65.14, 77.18)	76.28 (65.19, 84.26)	34.18 (20.06, 48.30)	37.66 (19.10, 56.23)	25.89 (14.93, 36.85)	20.92 (6.68, 35.16)	24.91 (11.42, 38.40)	41.23 (17.16, 65.30)	9.57 (0.00, 24.75)	22.44 (0.00, 47.11)

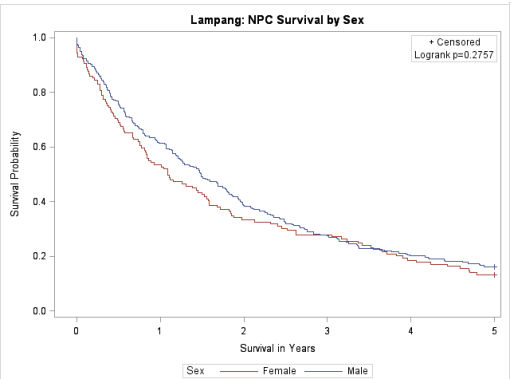
Figure 2.1: Joinpoint analyses of nasopharyngeal carcinoma by morphology and registry (APC [95% CI])

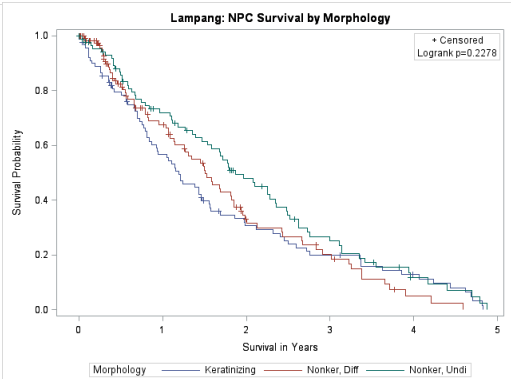


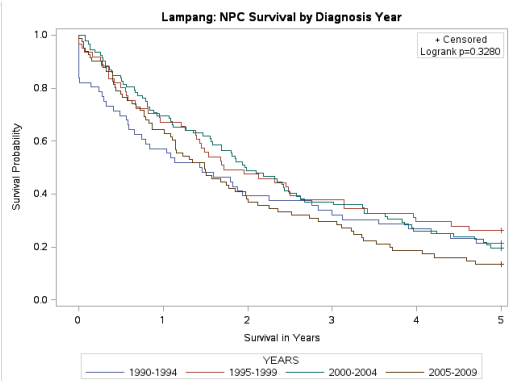
(*) denotes statistical significance at alpha = 0.05.

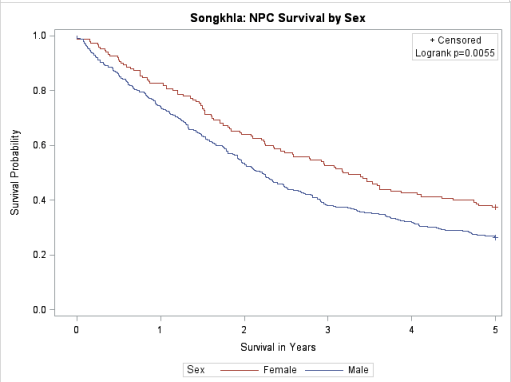
Figure 2.2: Kaplan–Meier Survival Curves of NPC Survival by Sex, Morphology and Year of Diagnosis

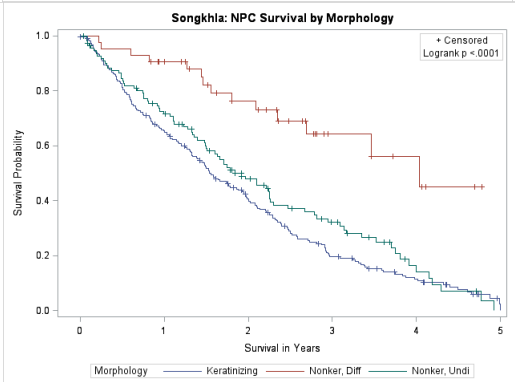


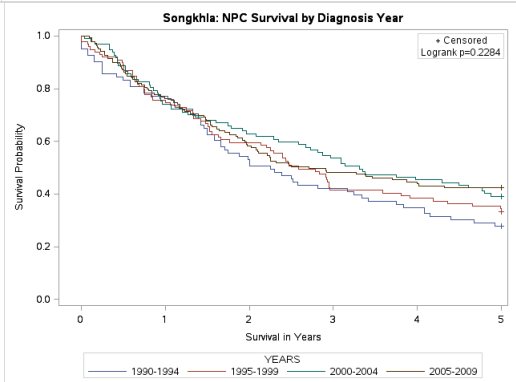

Lampang: NPC Survival by Sex
+ Censored
Logrank p=0.2757
Survival Probability
Survival in Years
Sex
Female
Male

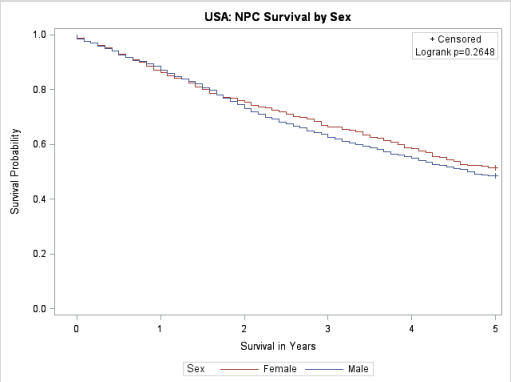

Lampang: NPC Survival by Morphology
+ Censored
Logrank p=0.2278
Survival Probability
Survival in Years
Morphology
Keratinizing
Nonker, Diff
Nonker, Undi

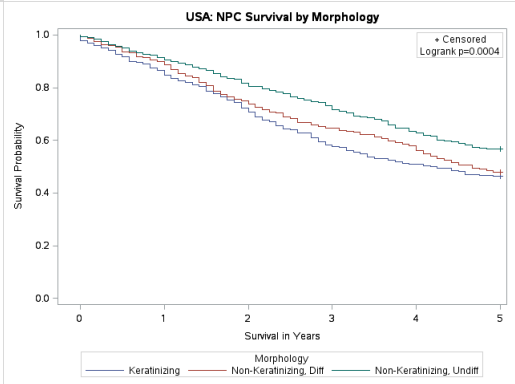

Lampang: NPC Survival by Diagnosis Year
+ Censored
Logrank p=0.3280
Survival Probability
Survival in Years
YEARS
1990-1994
1995-1999
2000-2004
2005-2009

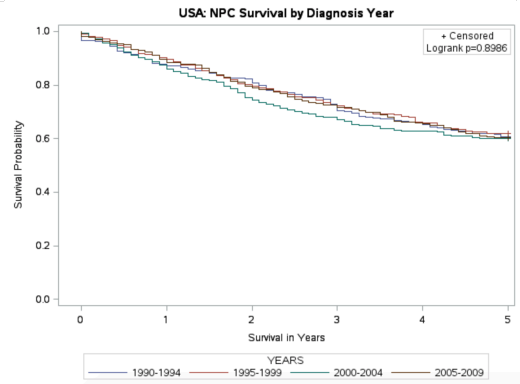

Songkhla: NPC Survival by Sex
+ Censored
Logrank p=0.0055
Survival Probability
Survival in Years
Sex
Female
Male


Songkhla: NPC Survival by Morphology
+ Censored
Logrank p <.0001
Survival Probability
Survival in Years
Morphology
Keratinizing
Nonker, Diff
Nonker, Undi


Songkhla: NPC Survival by Diagnosis Year
+ Censored
Logrank p=0.2284
Survival Probability
Survival in Years
YEARS
1990-1994
1995-1999
2000-2004
2005-2009


USA: NPC Survival by Sex
+ Censored
Logrank p=0.2648
Survival Probability
Survival in Years
Sex
Female
Male


USA: NPC Survival by Morphology
+ Censored
Logrank p=0.0004
Survival Probability
Survival in Years
Morphology
Keratinizing
Non-Keratinizing, Diff
Non-Keratinizing, Undiff


USA: NPC Survival by Diagnosis Year
+ Censored
Logrank p=0.8986
Survival Probability
Survival in Years
YEARS
1990-1994
1995-1999
2000-2004
2005-2009

Chapter 3

Increasing Incidence of EBV-related Nasopharyngeal Carcinoma in the United States

Abstract

Background: Incidence of nasopharyngeal carcinoma (NPC) has been historically low in the US. While etiological factors differ by histological subtype, Epstein-Barr virus (EBV) is accepted as the primary risk factor for non-keratinizing NPC. In light of the changing epidemiology of viral-associated cancers, it is important to evaluate temporal incidence of NPC in the US.

Methods: Incidence and survival data from 1973-2015 were obtained from the Surveillance, Epidemiology and End Results (SEER) Program. Stratified analyses were conducted to assess temporal trends in NPC by histological subtype, sex and race. The data were analyzed using SAS and Joinpoint Regression Software to determine age-adjusted incidence rates, trends in annual percent change (APC), and calculate 5-year relative survival estimates and Kaplan-Meier curves.

Results: Although overall NPC incidence is decreasing in the US, the non-keratinizing differentiated subtype is starkly increasing with an APC of approximately 4% among white males (95%CI: 2.5, 5.2), white females (95%CI: 1.9, 6.2), and black males (95%CI: 2.0, 5.7), 2.7% among black females (95%CI: 0.8, 4.6) and 1.8% among women of other race (95%CI: 0.4, 3.3). Racial disparities were noted, with 32% of non-keratinizing NPC cases among blacks occurred before the age of 40. Additionally, black males displayed consistently worse survival across all histological subtypes, while those individuals in the "other" race category, particularly females, experienced the highest 5-year relative survival estimates.

Conclusions: Our results indicate that the EBV-related differentiated NPC subtype is increasing across all genders and races in the US, with distinct incidence and survival disparities among blacks.

Introduction

Nasopharyngeal carcinoma (NPC) incidence varies greatly throughout the world. While historically rare in the United States, NPC is endemic to southeast China, Southeast Asia, the Middle East and North Africa [1]. Arising from the surface epithelium of the posterior nasopharynx [2], NPC is currently classified by the World Health Organization (WHO) into three major histological subtypes: keratinizing squamous cell carcinoma (WHO type 1), non-keratinizing carcinoma (WHO type 2), which can further be divided into differentiated and undifferentiated, and basaloid squamous cell carcinoma (WHO type 3) [3]. These pathological classifications have been repeatedly correlated with clinicopathologic features, treatment response and prognosis [4,5]. Basaloid squamous cell carcinoma is infrequent and thus is rarely reported in the literature [6]. Non-keratinizing NPC predominates in endemic regions, while the keratinizing subtype is most frequently observed in the non-endemic regions such as the United States (US) [7]. The unique geographic and ethnic distribution of NPC incidence throughout the world suggests a multifactorial etiology that involves viral, genetic and environmental components.

NPC incidence has been reported to rise monotonically with age in non-endemic countries, whereas in high-risk populations, such as southern China, incidence peaks between 45 and 54 years among both genders, implying that early exposure to carcinogens and Epstien-Barr virus (EBV) may result in younger onset NPC [8]. EBV, a herpesvirus most commonly known for causing mononucleosis, is generally accepted as the primary etiologic factor in non-keratinizing NPC. Increased latent EBV infection has been reported in dysplastic nasopharyngeal epithelium and NPC, suggesting that the infection occurs in the early stages of carcinogenesis [9-12]. Due to the ubiquitous nature of infection, and disproportionate global case distribution of NPC, several studies have assessed interactions between EBV and other lifestyle risk factors in high-risk populations. Dietary factors, namely early life exposure to Cantonese salted dried fish and other preserved foods containing volatile *N*-nitrosamines, have been implicated in NPC carcinogenesis through both the activation of latent EBV [13] and as independent carcinogens [14].

In non-endemic regions such as the United States, cigarette smoking and heavy alcohol consumption have been associated with keratinizing squamous cell carcinoma [15]. Compared to never smokers, heavy smokers have been reported to have a two- to four- fold increased risk of NPC [15-22]. Several studies have suggested that the effect conferred by smoking and alcohol primarily pertains to those diagnosed over age 50, suggesting a differing etiology among younger patients [15]. The increased prevalence of high-risk human papillomavirus (HPV) in oropharyngeal cancer etiology, paired with the similarities exhibited between the lymphoid and epithelium tissue of the oropharynx and nasopharynx, has led to investigation into the role of HPV in nasopharyngeal carcinogenesis. While results remain inconsistent, largely due to small sample sizes, there is evidence to suggest the role of HPV, and even HPV/EBV coinfection, in NPC [4,23-26].

It is important to evaluate incidence of NPC in the US over time in light of the changing epidemiology of HPV-associated oral cancers. Thus, in these analyses, we assess NPC incidence and mortality trends in the United States from 1973-2015 using the Surveillance, Epidemiology and End Results Program (SEER). In doing so, we aim to identify potential variation across race and sex and draw on the literature to make inferences on changing etiology over time.

Methods

Data Selection and Criteria

Cancer incidence and survival data were obtained from the Surveillance, Epidemiology and End Results (SEER) Program. For this analysis, SEER 9 data were used, which includes data from the following registries; Atlanta, Connecticut, Detroit, Hawaii, Iowa, New Mexico, San Francisco-Oakland, Seattle-Puget Sound and Utah registries. Data from 1973-2015 were available for all sites, with the exception of Seattle-Puget Sound and Atlanta, which joined in 1974 and 1975, respectively. Corresponding population estimates were obtained from SEER*Stat 8.3.5 [27] which are based upon the US Census Bureau's Population Estimates Program [28]. In addition to diagnosis, case information included: age, sex, race, date of diagnosis, SEER historical staging, histology, cause of death classification, and survival time in months.

Due to the small sample size of American Indians/Alaska Natives, this race group was combined with Asian/Pacific Islander to create the 'Other' race grouping.

Tumor site and histology were identified according to the International Classification of Diseases for Oncology (ICD-O-3) [6,29]. Histologic subtypes for NPC (Site codes: C11.0-C11.9) included: keratinizing squamous cell carcinoma (ICD-O histology codes 8070 and 8071), differentiated non-keratinizing carcinoma (ICD-O histology codes 8072 and 8073), undifferentiated non-keratinizing carcinoma (ICD-O histology codes 8020, 8021, and 8082) and 'carcinoma not otherwise specified' (ICD-O histology code 8010) [5]. In accordance with WHO guidelines, differentiated and undifferentiated non-keratinizing carcinomas were analyzed both separately and jointly to comprise the single non-keratinizing group. Overall NPC was defined as the culmination of all the aforementioned histological groupings. Basaloid squamous cell carcinomas (ICD-O histology code 8083) were excluded from these analyses due to small sample size (N=30). Additional exclusion criteria included unknown race, non-malignant carcinoma, and prior diagnosis of malignancy. Patient characteristics were assessed by histological subgrouping and further evaluation of age at diagnosis (in five year groupings) was conducted by visually displaying case frequencies by race for overall, keratinizing and non-keratinizing NPC.

Sex stratified exploratory analyses assessing incidence trends in extranodal NK/T-cell lymphoma, nasal type (ICD-O histology code 9719) were conducted for all effective years, 2001-2015, to further evaluate the potential role of EBV in US malignancies.

Incidence Trends

Age-adjusted incidence rates (standardized to the US population) and annual percent change (APC) were assessed using a log-linear model stratified by race, sex, and histological classification in Joinpoint Regression Program version 4.5.0.1 [30]. Joinpoint utilizes a Monte Carlo permutation method in order to assess number of joinpoints, slope in the trends, as well as their corresponding significance [31]. In circumstances where no cases were reported within a given year, a half-case was added to the age strata with the largest population to enable

computation on the log-linear scale [31,32]. SAS 9.4 software [27] was used to shape the data and generate descriptive statistics.

Survival Analysis

Individual-level data from the SEER*Stat case listing session was used to create Kaplan Meier curves in SAS 9.4 software. Graphs were gender specific, stratified by histologic type, and strata were compared using log-rank tests. Patients whose survival data were obtained from death certificates or autopsy only, as well as those with unknown survival duration, were excluded. In addition, those who cases with unknown age or those with a previous invasive cancer diagnosis were excluded from survival analyses. Five year relative survival, calculated as the ratio of the observed survival for NPC patients to the expected survival rate based on age, race, and calendar year, were computed using the Kaplan-Meier method in SEER*Stat. Expected survival life tables were constructed using the US Decennial Life Tables from the National Center for Health Statistics (NCHS) and from the US Annual Life Tables from NCHS for the years 2001–2014 [27].

Results

Between 1973 and 2015, 4,447 cases of NPC were reported among males and 1,838 among females in the US. Regardless of histological subtype, males accounted for ~70% of the overall cancer burden. In general, the peak incidence occurred at age 55 for both sexes, with the undifferentiated non-keratinizing carcinoma subgroup representing the youngest mean age at diagnosis (49.34 and 49.15 for males and females, respectively). Exploratory analyses revealed a bimodal distribution among blacks, with 25% of all cases being diagnosed under the age of 40, as compared to 12% and 21% of their white and other counterparts, respectively. While this trend was not apparent for keratinizing NPC, 32% of non-keratinizing NPC cases among blacks, 21% among whites and 22% among the other race group occurred under the age of 40 (**Figure 3.3**). The keratinizing squamous cell carcinoma subtype made up the majority of NPC cases, largely due to its predominance among white patients, who accounted for 49% of the overall sample size. Non-keratinizing NPC was diagnosed more frequently among blacks and predominated among the other race group. Among those with non-keratinizing NPC, the

undifferentiated cell type was more frequently reported across all races. SEER summary stage was available for cases diagnosed prior to 2004, and most cases were diagnosed with regional disease (**Table 3.1**).

While NPC incidence rates for the overall population appeared to have remained relatively stagnant among males (APC: -0.2 [95%CI: -0.4, 0.1]) and females (APC: -0.4 [95%CI: -0.8, 0.0]), these trends vary greatly across both race and histology. At the beginning of the study period, the expected age standardized rate (EASR) for white and black males were 0.67 and 0.66 per 100,000, respectively. White and black females both had notably lower rates, with EASRs of 0.34 and 0.16 per 100,000, respectively. The other race category, largely comprised of Asian/Pacific Islanders, exhibited the highest NPC rates, with an EASR of 4.79 and 1.76 per 100,000 among males and females, respectively. Temporal assessment of NPC incidence revealed significant decreases in white males (APC: -1.2 [95%CI: -1.5, -0.8]) and females (APC: -1.6 [95%CI: -2.1, -1.0]), black females (APC: -1.4 [95% CI: -2.5, -0.4]) as well as other males (APC: -1.0 [95%CI: -1.4, -0.6]) and females (APC: -1.3 [95%CI: -1.9, -0.7]) (**Table 3.2**).

These observed decreases in overall NPC are largely attributable to declines in the predominant keratinizing squamous cell carcinoma subtype. Joinpoint regression results for the overall population exhibited non-significant increases in keratinizing carcinoma among men from 1973-1985 (APC: 1.1 [95%CI: -1.2, 3.5]), followed by a significant decrease from 1985-2015 (APC: -2.6 [95%CI: -3.2, -2.1]); similar declines were observed among women (APC: -2.4 [95%CI: -3.0, -1.7]). While this pattern of reduced keratinizing NPC is seen across all races, it was not found to be significant among black males, and demonstrated the greatest impact among males (APC: -3.4 [95%CI: -4.1, -2.7]) and females (APC: -4.2 [95%CI: -5.1, -3.2]) of the other race category (**Table 3.2**).

Non-keratinizing NPC revealed two very different patterns in incidence trends, depending on histological subtype. Undifferentiated carcinomas significantly decreased with an APC of approximately -2% among white males (95%CI: -2.8, -1.3) and females and (95%CI: -3.0, -0.6), -2.7% among black females (95%CI: -4.2, -1.1), -1.5% among other males (95%CI: -2.2, -0.6) and -2.4% among women of other race (95%CI: -3.5, -1.4). The differentiated subtype, on the

other hand, increased starkly in incidence with an APC of approximately 4% among white males (95%CI: 2.5, 5.2), white females (95%CI: 1.9, 6.2), and black males (95%CI: 2.0, 5.7), 2.7% among black females (95%CI: 0.8, 4.6) and 1.8% among women of other race (95%CI: 0.4, 3.3). This dichotomy in incidence between differentiated and undifferentiated led to an attenuation in the overall trends of non-keratinizing NPC (**Table 3.2**).

Exploratory analyses on incidence trends in extranodal NK/T-cell lymphoma nasal type revealed very similar trends to those apparent in differentiated non-keratinizing NPC. Between 2001 and 2015, incidence increased among both sexes, with an APC of 3.2% (95%CI: 0.7, 5.8) among males and 3.9% (95%CI: 0.2, 7.8) among females. Race stratified analysis demonstrated even steeper increases among white males (APC: 3.8 [95%CI: 0.6, 7.1]) and females (APC: 4.1 [95%CI: 0.7, 7.6]). No significant increases were notes for the other race groups, likely due to small sample sized within these groups.

5-year relative survival estimated by race, sex and histological subtype can be found in **Table 3.3.** For all NPC types, 5 year relative survival was 53.0% among males, and 55.8% among females. **Figure 3.1** displays sex stratified Kaplan Meier survival plots by histological subgrouping. Both male and female patients with keratinizing squamous cell carcinoma had the worst survival when compared to the other histological subtypes (log rank $p<0.001$). 5-year relative survival rates for keratinizing squamous cell carcinoma were 41.9% and 41.5% for males and females of all races, respectively. While black males had consistently poorer survival across all histological subtypes, those individuals in the "other" race category, particularly females, experienced the highest 5-year relative survival estimates (**Table 3.3**).

Discussion

This is the first study, to our knowledge, to assess temporal changes in NPC incidence in the United States. Although overall NPC incidence appears to be decreasing in the US, the EBV-related differentiated non-keratinizing NPC subtype is increasing at a concerning rate among all race groups and genders in the US. NPC has long been considered a relatively rare disease in the US, with a reported incidence rate less than 1 per 100,000 [2,33,34]. Nevertheless, in endemic

regions, NPC remains a major public health problem, with annual incidence rates exceeding 20 per 100,000 individuals [33]. Several studies have previously reported decreased incidence of NPC in successive generations of Chinese migrants to the US [35]; however, the EASR among those belonging to the other race category, of which 98% identify as Asian/Pacific Islanders, remain over 5 times higher in females and 7 times higher among males, when compared to their white counterparts. Additionally, the rate of change in overall NPC incidence, represented as APC, between the white and other race individuals remained comparable throughout the study period, leading to continued disparity among this race group across time.

EBV, a prominent etiological factor in NPC, is a ubiquitous virus that infects and persists latently in over 90% of the global population [36]. Although primary infection is often subclinical, EBV replication can occur in oropharyngeal epithelial cells [37] as well as B lymphocytes in normal and malignant nasopharyngeal tissue [38]. EBV-encoded RNA has repeatedly been detected in both premalignant and malignant tissues of primarily non-keratinizing NPC[39-41]; nevertheless, few studies, particularly in non-endemic countries, have characterized the potential differences in etiology between differentiated and undifferentiated NPC. Antibody titers, particularly for IgA, have also been shown to not only precede cancer development [42], but to be associated with prognosis and recurrence [43]. A considerable amount of research has been conducted in order to determine whether particular strains of EBV may, at least in part, explain the international patterns of NPC [44-47]. Several different sequence variations have been detected in the oncogenic viral latent membrane protein 1 (LMP1) of EBV in NPC tumors [44,48-50], however, there remains no strong evidence to suggest an increased risk associated with different EBV variants [33,49-52]. In this study, we found differentiated non-keratinizing NPC incidence to be increasing among all sex and race groups, leading to speculation of an increased role of EBV in US NPC. Exploratory analyses into SEER incidence trends of extranodal NK/T-cell lymphoma, a form of non-Hodgkin's lymphoma consistently associated with EBV [53-67], demonstrated significant increases in this cancer type among both genders over this time period, further supporting the possibility for an increasing role in EBV etiology in US malignancies (**Figure 3.2**).

In certain endemic populations, such as the Cantonese, early age incidence peaks in NPC are generally associated with childhood consumption of, or even traditional weaning by [68], salted

fish and other preserved foods high in volatile *N*-nitrosamines [13]. Early-life infection by EBV [69,70], as well as genetic predisposition, have both been proposed as possible risk factors for early onset NPC, but little consensus has been reached regarding the etiology of such cases in the US. Although the average age at diagnosis in our study was 55 among both sexes, we noted a bimodal distribution in cases among blacks, with 25% of patients being diagnosed under the age of 40 as compared to 12% of their white counterparts. While this distribution has been observed previously [35], we noted this disparity to be particularly apparent in the non-keratinizing subtype, where 32% of cases among blacks occurred before the age of 40 (**Figure 3.3**). Though we are unable to definitively conclude that the increase in these young onset cases is due to EBV alone, EBV seroprevalence has been reported to be significantly higher among African-American youth when compared to their white peers [71,72]. Further studies are needed in order to properly assess the role childhood infection by EBV, as well as possible gene-environment interactions in the US population.

The majority of case control studies report a 2- to 4-fold increased risk in NPC among smokers [15,16,18-22]. Vaughan et al. estimated that two thirds of keratinizing NPC in the US are attributable to smoking, while both subtypes of non-keratinizing carcinoma failed to demonstrate significant associations [15]. Decreases in smoking prevalence in the US [73] may explain the decreases in keratinizing squamous cell carcinoma seen in our analyses. Diet, which has been the primary nonviral exposure associated with NPC in endemic regions, has been postulated to impact NPC primarily thought the consumption of preformed nitrosamines or nitrosamine precursors [74]. While cultural differences in dietary patterns preclude early and repeated exposure to salted fish [75], harissa, quaddid and touklia [76] from US dietary etiology, preserved meats, which contain high levels of added nitrites, have been studied and found not to be significantly associated with NPC carcinogenesis [74]. Although this particular study did not assess genetic variation, individuals processing the c2/c2 metabolic genotype for CYP2E1, a catalytic enzyme for the metabolic activation of low-molecular weight nitrosamines, have been shown to experience a 2.6-fold increased risk of NPC when compared to those possessing one or two copies of the wild-type allele [77]. Moreover, alcohol, in addition to its innate role as a carcinogen [15,18], has been shown in animal models to increase the carcinogenicity of ingested nitrosamines [78,79], further stressing the importance of dietary interaction studies in the understanding of nasopharyngeal carcinogenesis.

Our study confirmed previous reports of a survival advantage of the undifferentiated non-keratinizing morphology over the differentiated non-keratinizing and keratinizing squamous cell NPC [5,35]. Additionally, we found that the 'other' racial grouping had consistently improved survival when compared to white and black patients, particularly among those presenting with keratinizing squamous cell carcinoma; these findings are consistent with other reports of a survival advantage among Chinese/Asian patients [5,35,80]. One proposed rationale for this observation may be the high proportion of polymorphisms in the epidermal growth factor receptor (EGFR) found in the Chinese/Asian population, leading to lower EGFR expression, and consequently improved prognosis [5,81-84].

There are several limitations present in this study. Although the use of SEER-9 allows for temporal analysis of cancer trends since 1973, it represents only half of the participating registries to date. Additionally, population estimates used for trends analyses were only available for the White, Black and Other race categories until 1990, resulting in our inability to assess age-adjusted NPC incidence trends in specific other races (American Indian/Alaska Native and Asian/Pacific Islander) or by Hispanic ethnicity for the entire study period. Treatment variables, particularly those pertinent to NPC such as chemotherapy and radiation, are not suggested for use by SEER due known data gaps for these variables. The absence of data regarding comorbidities, smoking, drinking behavior, and EBV prevalence, limits our ability to make definitive statements regarding the underlying etiology influencing the observed trends. Although more precise treatment and comorbidity variables are available by linking the SEER database to the Medicare claims database, the majority of NPC patients in this study are too young to qualify for Medicare (<65 years old). Trends in adenocarcinoma of the nasopharynx, largely associated with occupational exposures such as wood dust [85-87], were not able to be assessed due to small sample sizes. Finally, the WHO classification system for NPC had changed several times throughout the study period, consequently leading to concerns regarding the interpretability of subtype specific results. That being said, the changes within the WHO guidelines were made in the grouping of the subtypes, not the pathology; this study utilizes morphology codes to define NPC subtypes, and therefore the changes in WHO classifications have no effect on the study nor the conclusions.

Although NPC remains relatively rare in the overall US population, the EBV-related differentiated subtype is increasing across all genders and races. Many studies assessing non-keratinizing NPC, particularly in the US, aggregate differentiated and undifferentiated carcinoma due to lack of sample size; the results of this study highlight the need for additional research to elucidate the potential etiological differences between these histological subgroups, consequently addressing the rationale for the disparity in the observed incidence trends. Due to the fact that prospective cohort studies with comprehensive exposure assessment would require decades to recruit a sufficient sample size to assess gene-environment interactions, multicenter studies or case-control studies in high incidence regions may prove to be a more feasible method to improve understanding of NPC etiology. Additionally, studies evaluating the biological mechanism by which EBV is involved in NPC are needed in order to move EBV preventative and targeted therapeutic methods forward, such as immunotherapy or even an EBV vaccine. Recent interest into the possible role of HPV in NPC, likely representing subepithelial extension from the oropharynx due to the lack of anatomical constraints in Waldeyer's ring [1], may also aid in understanding incidence trends and NPC pathogenesis. Finally, comprehensive characterization of risk factors among young adults is needed in order to reveal the cause for the adolescent incidence peak observed in African American populations in this study.

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Table 3.1: Nasopharyngeal Carcinoma Distribution, 1973-2015, by Sex and Histology

	Overall Nasopharyngeal Carcinoma		Keratinizing Squamous Cell Carcinoma		Differentiated Non-Keratinizing Carcinoma		Undifferentiated Non-Keratinizing Carcinoma		Carcinoma NOS	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number of Cases, N (%)	4447 (70.76)	1838 (29.24)	1979 (70.30)	836 (29.70)	710 (73.35)	258 (26.65)	996 (70.49)	417 (29.51)	762 (69.97)	327 (30.03)
Mean age (SD)	54.55 (15.00)	55.36 (16.47)	57.35 (13.65)	59.24 (14.76)	53.91 (13.24)	54.18 (16.30)	49.34 (16.28)	49.15 (16.45)	54.69 (16.22)	54.31 (18.02)
Race										
White	2175 (48.91)	893 (48.59)	1247 (63.01)	568 (67.94)	268 (37.75)	68 (26.36)	361 (36.24)	143 (34.29)	299 (39.24)	114 (34.86)
Black	426 (9.58)	163 (8.87)	209 (10.56)	69 (8.25)	60 (8.45)	28 (10.85)	85 (8.53)	41 (9.83)	72 (9.45)	25 (7.65)
Asian/Pacific Islander	1813 (40.77)	761 (41.40)	510 (25.77)	194 (23.21)	377 (53.10)	156 (60.47)	543 (54.52)	227 (54.44)	383 (50.26)	184 (56.27)
American Indian/Alaska Native	33 (0.74)	21 (1.14)	13 (0.66)	5 (0.60)	5 (0.70)	6 (2.33)	7 (0.70)	6 (1.44)	8 (1.05)	4 (1.22)
Stage										
Localized	388 (8.72)	175 (9.52)	202 (10.21)	103 (12.32)	47 (6.62)	12 (4.65)	91 (9.14)	43 (10.31)	48 (6.30)	17 (5.20)
Regional	1786 (40.16)	759 (41.29)	848 (42.85)	366 (43.78)	235 (33.10)	90 (34.88)	441 (44.28)	180 (43.17)	262 (34.38)	123 (37.61)
Distant	493 (11.09)	190 (10.34)	281 (14.20)	102 (12.20)	54 (7.61)	10 (3.88)	100 (10.04)	52 (12.47)	58 (7.61)	26 (7.95)
Unstaged	247 (5.55)	132 (7.18)	122 (6.16)	75 (8.97)	11 (1.55)	4 (1.55)	47 (4.72)	19 (4.56)	67 (8.79)	34 (10.40)
Cases diagnosed 2004+	1533 (34.47)	582 (31.66)	526 (26.58)	190 (22.73)	363 (51.13)	142 (55.04)	317 (31.83)	123 (29.50)	327 (42.91)	127 (38.84)

Table 3.2: Joinpoint Analyses of Nasopharyngeal Trends by Sex and Morphology [APC (CI)]

	All		White		Black		Other	
	Male	Female	Male	Female	Male	Female	Male	Female
Overall Nasopharyngeal	-0.2 (-0.4, 0.1)	-0.4 (-0.8, 0.0)	-1.2* (-1.5, -0.8)	-1.6* (-2.1, -1.0)	-0.1 (-1.0, 0.7)	-1.4* (-2.5, -0.4)	-1.0* (-1.4, -0.6)	-1.3* (-1.9, -0.7)
Keratinizing	1.1 (-1.2, 3.5)ˠ -2.6* (-3.2, -2.1)¥	-2.4* (-3.0, -1.7)	-2.1* (-2.6, -1.6)	-2.4* (-3.2, -1.6)	-1.2 (-2.4, 0.0)	-2.3* (-4.1, -0.4)	-3.4* (-4.1, -2.7)	-4.2* (-5.1, -3.2)
Non-Keratinizing	1.0* (0.5, 1.5)	1.2* (0.5, 1.8)	0.5 (-0.2, 1.1)	-0.0 (-1.1, 1.1)	0.6 (-0.8, 2.0)	-0.6 (-2.1, 0.9)	-0.8* (-1.5, -0.2)	-0.6 (-1.5, 0.2)
Differentiated	3.0* (2.1, 3.9)	4.4* (3.0, 5.9)	3.8* (2.5, 5.2)	4.0* (1.9, 6.2)	3.9* (2.0, 5.7)	2.7* (0.8, 4.6)	-0.1 (-1.3, 1.0)	1.8* (0.4, 3.3)
Undifferentiated	-0.5 (-1.1, 0.1)	-0.8* (-1.5, -0.0)	-2.1* (-2.8, -1.3)	-1.8* (-3.0, -0.6)	-1.6 (-3.3, 0.2)	-2.7* (-4.2, -1.1)	-1.5* (-2.2, -0.6)	-2.4* (-3.5, -1.4)

*** denote statistical significance at α=0.05**
ˠ 1973-1985
¥ 1985-2015

Table 3.3: Five-year Relative Survival Estimates by Sex and Race [% (CI)]

	All		White		Black		Other	
	Male	Female	Male	Female	Male	Female	Male	Female
Cell Type, % (CI)								
Overall Nasopharyngeal	53.0 (51.3, 54.7)	55.8 (53.2, 58.4)	47.7 (45.2, 50.2)	45.7 (41.8, 49.6)	44.4 (38.7, 49.9)	56.5 (47.1, 64.8)	60.8 (58.2, 63.2)	66.1 (62.2, 69.7)
Keratinizing	41.9 (39.4, 44.4)	41.5 (37.6, 45.4)	37.7 (34.6, 40.9)	37.1 (32.3, 41.8)	35.5 (27.7, 43.4)	35.9 (22.9, 49.0)	53.2 (48.3, 57.7)	54.3 (46.3, 61.6)
Non- Keratinizing	64.7 (62.0, 67.2)	67.7 (63.5, 71.6)	64.4 (59.8, 68.6)	61.0 (52.7, 68.3)	54.4 (44.4, 63.3)	70.6 (55.8, 81.2)	66.4 (62.8, 69.7)	70.5 (65.1, 75.2)
Differentiated	63.1 (58.7, 67.1)	63.4 (56.0, 69.9)	62.3 (55.0, 68.7)	51.6 (36.2, 65.0)	49.5 (32.8, 64.2)	66.5 (41.2, 82.9)	65.3 (59.4, 70.5)	67.3 (58.1, 74.9)
Undifferentiated	65.8 (62.4, 69.0)	70.1 (64.8, 74.8)	65.8 (59.7, 71.1)	65.0 (55.1, 73.3)	57.1 (44.5, 67.9)	72.6 (53.2, 85.0)	67.1 (62.5, 71.3)	72.5 (65.6, 78.3)
NOS	54.6 (50.4, 58.6)	66.2 (59.7, 71.9)	51.9 (45.0, 58.4)	58.6 (46.5, 68.9)	47.7 (34.3, 59.9)	69.1 (42.9, 85.1)	57.5 (51.8, 62.9)	69.9 (61.6, 76.7)

Figure 3.1: Kaplan Meier Survival Curves for Overall Survival by Sex and Histology

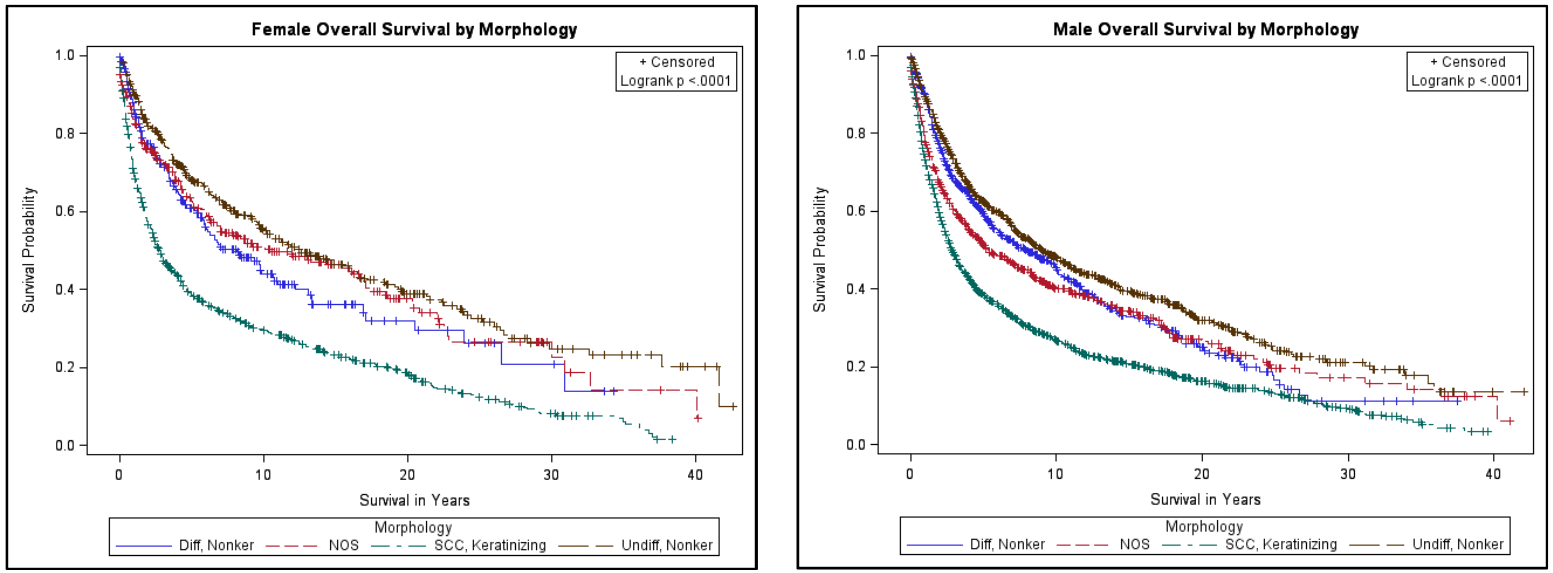
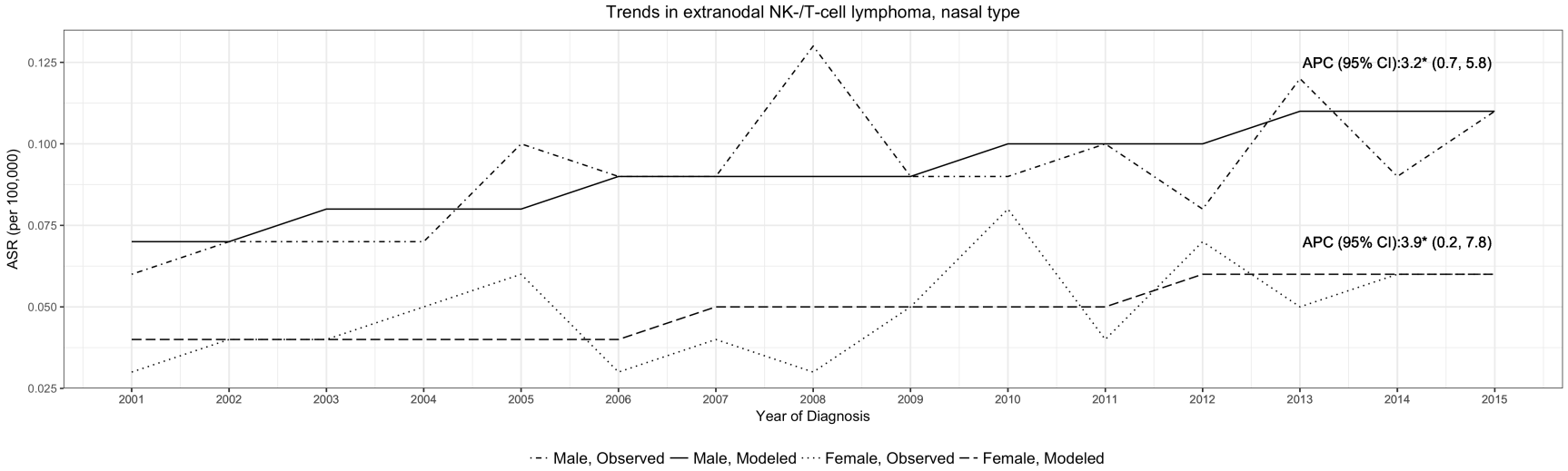
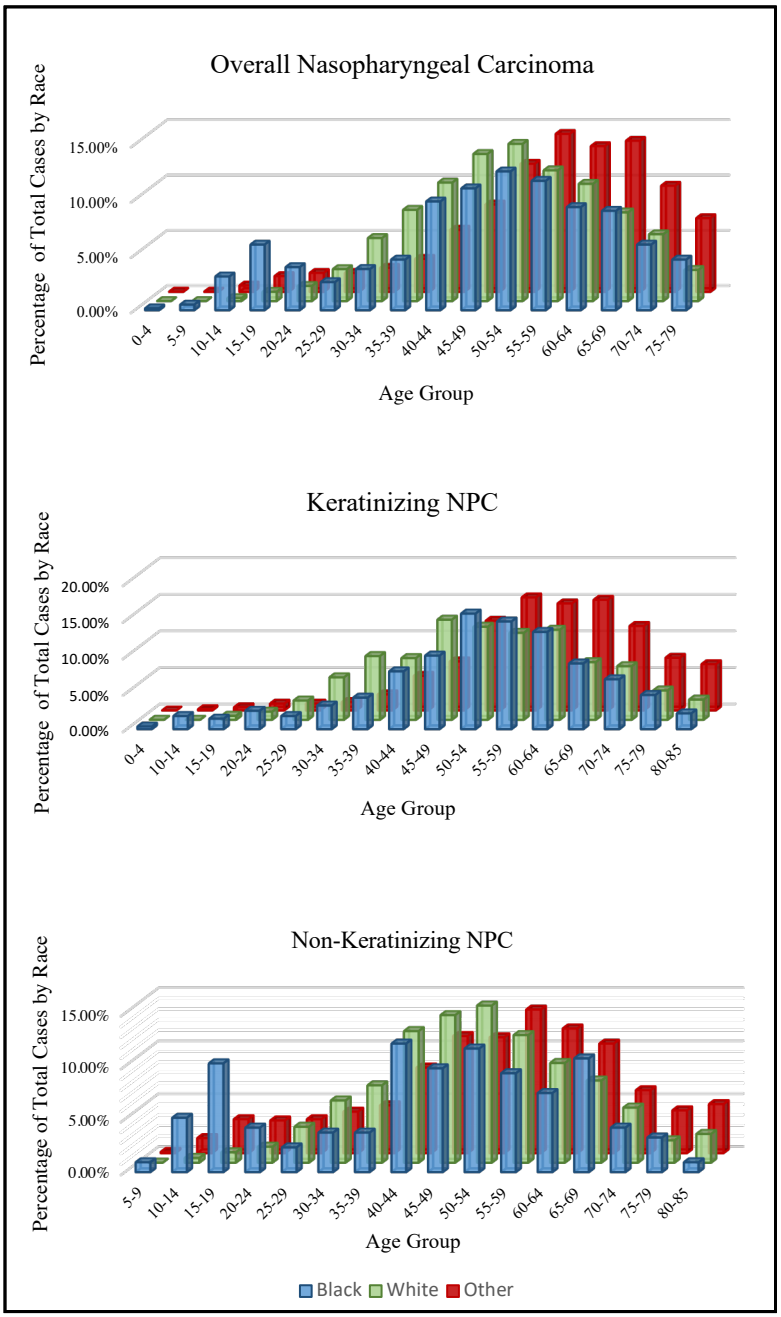


Figure 3.2: Incidence Trends in Extranodal NK/T-cell Lymphoma, Nasal Type (2001-2015)



	White		Black		Other	
Sex	Male	Female	Male	Female	Male	Female
N (%)	397 (45.6)	226 (26.0)	34 (3.9)	11 (1.3)	118 (13.6)	85 (9.8)
APC (95% CI)	3.8* (0.6, 7.1)	4.1* (0.7, 7.6)	0.6 (-6.2, 8.0)	3.9 (-4.2, 12.8)	0.4 (-4.6, 5.7)	1.8 (-3.6, 7.4)

Figure 3.3: Age Distribution of Nasopharyngeal Carcinoma Cases by Race and Subtype, SEER 1973-2015



Chapter 4

Decreasing Rates of Head and Neck Cancer in Southeast, but not East, Asia Follow Trends in Tobacco Control

Abstract

Background: Head and neck cancer (HNC) is the sixth most common cancer in the world, with the largest burden occurring in developing countries. Although the primary risk factors have been well characterized, little is known about the temporal trends in HNC across Asian countries.

Methods: Cancer cases from three Thai provinces (Songkhla, Chiang Mai, Lampang), Singapore, Manila, Shanghai, and the US were selected by ICD-10 code from the IARC CI5*plus* database for the following sites: oral cavity (00, 03-06), tongue (01-02), pharynx (09-10, 12-14), and larynx (32). The data were analyzed using R software (3.3.3) and Joinpoint Regression Software (4.5.0.1) to determine age-standardized incidence rates (ASR) and trends of annual percent change (APC).

Results: Males had higher incidence rates of HNC than females across all registries. Among females, the highest baseline rates were observed in Manila (ASR: 9.09 per 100,000) and the lowest in Shanghai (ASR: 1.85 per 100,000). Among males, the highest baseline incidence rates were seen in the US (ASR: 21.44 per 100,000) and the lowest in Shanghai (ASR: 5.79 per 100,000). Incidence is decreasing everywhere except among Shanghai males from 1998 and 2002 (APC: 7.87; [95%CI: 1.1, 15.1]). Subsite analyses reveal increases in male laryngeal cancer (APC: 9.10 [95%CI: 2.2, 16.4]) contributing to Shanghai's upward trend.

Conclusions: Largely in concurrence with strong tobacco control policies, HNSCC rates appear to be decreasing across the US and Southeast Asia; however, dangerously high smoking rates in Shanghai are likely attributable for Shanghai's concerningly increasing laryngeal cancer rates.

Introduction

Head and neck cancer (HNC) is the sixth most common malignancy worldwide and is comprised of several principal subsites, including the oral cavity, pharynx, larynx and nasal cavity [1,2]. Globally, HNC accounted for more than 650,000 cases and 330,000 deaths in 2018 [3]. More than 90% of head and neck neoplasms are squamous cell carcinomas (HNSCC) [4], and nearly 40% of HNSCCs arise from the epithelium of the mucosal lining of the oral cavity [3]. While HNSCC accounts for 5-10% of all new cancer cases in North America and Europe [5], geographic differences, such as the population age structure, prevalence of established risk factors and access to screening and care, all play a key role in making HNSCC a behaviorally heterogeneous and therapeutically challenging disease. Although incidence rates differ by subsite, the greatest burden of head and neck cancer is observed in low and middle-income countries [6,7]. Globally, the highest incidence of oral cavity cancer (OCC) is in Melanesia with an age standardized rate (ASR) of 22.9 for males and 16.0 for females, followed by South-Central Asia with an ASR of 9.9 for males and 4.7 for females [8]. Nevertheless, regardless of geography, incidence rates of head and neck cancer are significantly higher for men than women worldwide, with subsite-specific male to female ratios up to 10:1 [9-12].

The wide geographical variation in HNSCC incidence can primarily be attributed to demographic differences in established risk behaviors, such as the use of alcohol and tobacco consumption (both smoked and smokeless). The habit of chewing betel quid, consisting of betel leaf, areca nut, slaked lime, and occasionally tobacco, has predominantly been thought to account for the high incidence of OCC in Asian countries [11,13,14]. As the prevalence of these known risk factors change, they consequently influence trends in HNC. This phenomenon has recently been observed in the United States (U.S.) and other high-income countries, where decreasing incidence of oral and laryngeal squamous cell carcinomas, corresponding to declines in the use of tobacco products [15], have led to the detection of an epidemiologic shift that has elucidated the role of high-risk human papillomavirus (HPV) strains in head and neck cancer pathology [16,17].

While numerous studies have been conducted to assess the changing etiology of HNSCC in the western world, little is known about HNC behavior in high incidence areas, such as East and

Southeast Asia. To provide an overview of the temporal changes to HNSCC in these regions, we utilized IARC's Cancer Incidence in Five Continents database to assess subsite specific trends across three Thai registries (Chiang Mai, Lampang and Songkhla), Shanghai (China), Singapore, Manila (Philippines) and compare them to the U.S.

Methods

Head and neck cancer incidence rates aggregated over 5-year age groups (0-85+) by year, sex, subsite and cancer registry were obtained from IARC's Cancer Incidence in Five Continents Time Trends Database (CI5-Plus). The CI5 series of databases are comprised of high-quality data obtained in order to assess annual global cancer incidence [18]. In this study, we restricted our analyses to six cancer registries in East and Southeast Asia including: Manila (1983-2002), Singapore (1980-2007), Shanghai (1988-2002), Lampang (1993-2007), Songkhla (1993-2007), Chiang Mai (1983-2002) and compared them to those in the United States (1975-2007).

Cases included confirmed malignant neoplasms within the head and neck as defined by the International Classification of Diseases codes for oncology (ICD-O). Sites of interest included the oral cavity, tongue, pharynx and larynx. Oral cavity sites included the lip (C00.0-C00.6, C00.8-C00.9), gum (C03.0-C03.1, C03.9), floor of the mouth (C04.0-C04.1, C04.8-C04.9), palate (C05.0-C05.2, C05.8-C05.9), and other parts of the mouth (C06.0-C06.2, C06.8-C06.9). Tongue included the base of the tongue as well as other and unspecified parts of the tongue (C01, C02.0-C02.4, C02.8-C02.9). Pharynx sites included the tonsil (C09.0-C9.1, C09.8-C09.9), oropharynx (C10.0-C10.4, C10.8-C10.9), pyriform sinus (C12), hypopharynx (C13.0-C13.2, C13.8-C13.9), and other and ill-defined sites in the lip, oral cavity and pharynx (C14.0, C14.2, C14.8). All laryngeal sites were used (C32.0-C32.3, C32.8-C32.9) and overall HNSCC incidence was calculated as the sum across all subsites. Nasal cavity (C30.0), nasopharyngeal (C11.0-C11.3, C11.8-C11.9) and salivary gland (C07, C08.0-C08.1, C08.9) cancers were excluded due to differing etiology [19,20].

Joinpoint Regression Program version 4.5.0.1 was used to assess trends under a log-linear model and to compute the annual percent change (APC) in age-standardized incidence rates [21]. The aforementioned program utilizes a Monte Carlo permutation method in order to assess number of

joinpoints, slope in the trends and their significance [22]. When no cases were present within a given year, a half-case was added to the age strata with the largest population to enable computation on the log-linear scale [21]. Population denominators were similarly obtained from CI5-Plus by 5-year age grouping for each year and Segi (1960) standardization was applied [23]. R-statistical software 3.3.3 was used to shape the data and Joinpoint outputs were used to generate plots.

Results

HNSCC cases were predominantly male across all registries, with the greatest disproportion observed in Songkhla and Singapore, where males accounted for ~80% of all reported cases within their respective registries. While age at diagnosis was not available for those >80 years old within the Songkhla, Chiang Mai and Manila registries, the majority of HNSCC cases occurred between the ages of 61 and 80 across all study sites. Among men, laryngeal cancer accounted for the highest proportion of cases across all the registries except Songkhla, where pharyngeal cancer made up just over 30% of reported cases. Among women, oral cavity cancer was the primary contributor to head and neck cancer burden across all study sites except Singapore, where there was a slight observed predominance of tongue cancer (**Table 4.1**).

At baseline, the U.S. was found to have the highest overall HNSCC incidence among men, with an expected age standardized rate (EASR) of 21.44 per 100,000. Although much lower than that among males, Manila stood out as having the highest HNSCC burden among females, with an EASR of 9.09 per 100,000. Overall, these trends appear to be decreasing across both genders and all registries except Shanghai males, where there is an observed, statistically significant increase in HNSCC cancer burden between 1998 and 2002 (APC: 7.87 [95%CI: 1.1, 15.1]). While the majority of these overall decreases are modest, with Shanghai and Lampang females as well as both sexes in Songkhla failing to reach statistical significance, a drastic decrease in HNSCC was noted among Filipino females, where the EASR dropped from 6.68 per 100,000 in 1997 to 3.14 per 100,000 in 2002 (APC: -13.8 [95%CI: -19.9, -7.1]) (**Figure 4.1**).

With an EASR of 7.30 per 100,000, the U.S. boasted the highest laryngeal cancer rates among men at baseline; nevertheless, with consistent decreases at a magnitude of 1.2% (95%CI: -2.1, -

0.4) between 1980 and 1990, and 3.1% (95%CI: -3.7, -2.4) between 1990 and 2002, this rate dropped to 4.45 per 100,000 by 2002. Decreases in laryngeal cancer incidence were also observed among males in Lampang (APC: -6.3 [95%CI: -9.5, -3.0]), Chiang Mai from 1998-2002 (APC: -19.7 [95%CI: -34.2, -2.0]), Singapore (APC: -2.5 [95%CI: -3.1, -1.9]), and Manila from 1995-2002 (APC: -5.1 [95%CI: -9.1, -0.9]). While male trends appear to have remained stagnant in Songkhla, Shanghai was the only registry in which laryngeal cancer incidence increased, with an APC of 9.1% (95%CI: 2.2, 16.4). When compared to those amid males, female laryngeal cancer incidence rates appeared to be much lower, with Chiang Mai having the highest EASR of 2.92 per 100,000 at baseline. Temporal trends demonstrated significant decreases in laryngeal cancer in Chiang Mai (APC: -8.0 [95%CI: -11.7, -4.2]), Singapore (APC: -5.6 [95%CI: -7.2, -4.1]), Manila (APC: -2.6 [95%CI: -4.4, -0.7]), Shanghai (APC: -4.3, [95%CI: -7.9, -0.7]), and the U.S. from 1989-2002 (APC: -2.7 [95%CI: -4.1, -1.3]) (**Figure 4.2**).

While oral cavity rates at baseline were highest among U.S. males, with an EASR of 6.82 per 100,000, among females, the highest rates were found to be in Manila, with an EASR of 4.40 per 100,000. Significant decreases in incidence were noted among males in Chiang Mai (APC: -2.8 [95%CI: -4.5, -1.0]), Singapore (APC: -2.7 [95%CI: -3.8, -1.7]), Manila (APC: -3.8 [95%CI: -5.3, -2.4]), Shanghai (APC: -2.7 [95%CI: -4.6, -0.7]), and the U.S. (APC: -3.5 [95%CI: -3.8, -3.2]). Similarly, among females, significant reductions in oral cavity cancer burden were found in Singapore (APC: -2.9 [95%CI: -4.2, -1.7]), Manila (APC: -5.4 [95%CI: -6.9, -3.8]) and the U.S. (APC: -2.0 [95%CI: -2.3, -1.7]) (**Figure 4.2**).

When compared to other registries, Songkhla males and Filipino females were found to have the highest EASR for both pharyngeal (4.88 and 1.64 per 100,000, respectively) and tongue cancer (3.74 and 2.55 per 100,000, respectively). While cancer incidence among both of these subsites have remained stagnant in Songkhla, decreases in pharyngeal cancer were noted among Lampang males and females (APC: -5.7 [95%CI: -9.2, -2.1]; APC: -6.8 [95%CI: -13.0, -0.1], respectably), Chiang Mai males and females (APC: -3.4 [95%CI: -5.6, -1.3]; APC: -4.8 [95%CI: -8.2, -1.3], respectably), Singapore males and females (APC: -1.1 [95%CI: -1.9, -0.3]; APC: -2.5 [95%CI: -4.7, -0.4], respectably), U.S. males and females (APC: -0.9 [95%CI: -1.3, -0.5]; APC: -2.7 [95%CI: -3.2, -2.1], respectably) and, most notably, Filipino females for 1996-2002 (APC: -

16.0 [95%CI: -27.9, -2.1]). Conversely, incidence trends for tongue cancer appear to be much more variable, with wide fluctuations in rates leading to largely null results. Nevertheless, significant decreases were noted among Chiang Mai females (APC: -5.2 [95%CI: -8.7, -1.5]) and Filipino females (APC: -4.0 [95%CI: -6.4, -1.6]) (**Figure 4.2**).

Discussion

In this study, we found HNSCC to be decreasing across Thailand, Singapore, Manila and the US, but increasing among Shanghai males. While policies around relevant risk behaviors vary across these study sites, general decreases in smoking and betel quid use in Southeast Asia are likely attributable to the favorable cancer trends within this region. Nevertheless, largely stagnant temporal incidence rates in tongue cancer across these registries point to research opportunities in cancer etiology within this subsite.

Tobacco use, whether it be in chewing or combustible form, has long been accepted as the leading risk factor for head and neck cancer [1,24]. HNSCC rates among smokers have been estimated to be approximately ten times that of never smokers, with risk estimates increasing in a dose-dependent manner based upon duration and extent of smoking [15]. While this association is strongest for laryngeal cancer, use of combustible tobacco has also been shown to increase the risk of hypopharyngeal, oropharyngeal and oral cavity cancer development [25]. Smoking cessation has been shown to reduce cancer risk, but ever-smokers continue to have a greater likelihood of developing HNSCC when compared to never-smokers [26]. Additionally, alcohol consumption, while being an independent risk factor for HNSCC, also has a synergistic multiplicative effect when combined with tobacco use [1,9,24,27]. Both tobacco and alcohol consumption have historically been much more common among men than women, leading to the male predominance seen within HNSCC [28,29]. While the World Health Organization (WHO) reports progressive decreases in smoking rates in the U.S., Singapore, Thailand, the Philippines and Singapore [30-32], alcohol consumption has increased dramatically in the past 50 years across all study sites except the U.S. [28,29,33,34].

Tobacco history in the US can largely be characterized by its widespread adoption in the first half of the 20th century and its control thereafter. Particularly in recent years, rising public

awareness and support for tobacco control efforts have markedly influenced smoking rates, with adult 'current smoker' prevalence down from 42.5% in 1965 to 20.9% in 2005 [35-37]. These reductions are reflected in HNSCC rates, particularly within laryngeal cancer, where incidence rates decreased among both males and females. Although the international trade of tobacco is a substantial income source for Singapore, the government has demonstrated effective control over the domestic use of tobacco. Initially introduced in 1970 to control smoking in public places, Singapore has since expanded its legislation to include national control policies that encompass educational reform, taxation, intersectoral collaboration and community participation [38]. While smoking prevalence rates among females have historically been low, fluctuating around 3%, rates among males dropped from 37% in 1984 to 27% in 1998 [38]; these decreases were consequently reflected within the decreasing laryngeal cancer rates among both sexes. Likewise, Thailand is known for having some of the strongest tobacco control policies in Asia [39,40], with numerous regulations including health warnings, taxation, bans on advertisement and smoking in many public spaces [41,42]. Due in large part to these efforts, the smoking rate in Thailand has decreased from 30% in 1976 to 19.9% in 2013 [15]; subsequently, these behavioral modifications have led to significant decreases in laryngeal cancer among Lampang and Chiang Mai males, as well as Chiang Mai females. In contrast, Manila has essentially no regulations on advertisement or sale of tobacco, minors are able to buy and sell products, and the taxation rate is considerably lower than is recommended by the WHO [43]. The National Coalition on Tobacco control was established in 1988, but this has done little to reform tobacco legislation; nevertheless, smoking rates have been reported to be decreasing [44]. Although Manila's laryngeal cancer rates remain among the highest when compared to our other study sites, incidence is decreasing among both Filipino males and females. Finally, China is the world's largest tobacco manufacturer and accounts for about 30% of the world's cigarette consumption. While Shanghai has set forth regulations on smoking in certain public areas, as well as initiated a project aimed at improving cessation clinics [45], reports from 2005 and 2010 indicated that 74% and 84% of smokers in China, respectively, had no desire to and have never thought about quitting smoking [45,46]. Although both smoking and laryngeal cancer rates appear to be decreasing among women, Shanghai was the only registry to demonstrate significant increases in male laryngeal cancer incidence rates, with an APC of 9.1% (95%CI: 2.2, 16.4) between 1998 and 2002.

In the US, outside of the aforementioned risk behaviors, the use of smokeless tobacco in the form of chewing tobacco, wet snuff, or dried out snuff are the primary etiological factors associated with OCC [47]. The decreased popularity of these products [48] has led to corresponding decreases in oral cavity cancer among both males and females in the US. Throughout Asia, betel quid, defined as sliced or processed areca nut wrapped in the leaves of the piper betel plant and mixed with slaked lime (calcium hydroxide), is often chewed in social or religious settings [49]. Tobacco is only included in some uses of betel nut, but studies have shown that both with and without tobacco, chewing betel nut leads to an increased risk of OCC [49-51]. In Thailand, overall betel nut usage has been declining, and is now predominantly seen in the older and more rural populations [52-54]. Although significant decreases in OCC were only observed among Chiang Mai males, incidence data suggests decreases across all Thai study populations with the exception of males in rural Lampang. Manila is also seeing a decrease in OCC, likely corresponding to decreases in betel nut usage with successive generations and thought to be associated with migration out of rural communities, attainment of higher education and economic status [13]. The decreasing OCC rates in Singapore can likely be attributed to the anti-spitting laws, which have indirectly discouraged the use of betel-quid chewing in the general population [55]. Although little is known about betel quid use in mainland China, the existing literature points to the habit being localized to the southern provinces of Hunan, Hainan and Yunnan [56-59]. Consequently, the decreases in OCC observed among Shanghai males are likely more reflective of changes in traditional combustible tobacco and alcohol risk factors; however, several studies have previously questioned the data quality of China's registries, particularly in regards to the escalating tobacco epidemic in this region and low reported incidence of OCC [60,61].

High risk human papillomavirus (HPV) strains have been shown to be an independent risk factor for head and neck cancer and are found primarily in the oral cavity and oropharynx [9,10,62]. The burden of HPV related oral cancers appears to be increasing in developed countries, likely due to changes in sexual behaviors, but corresponding data is largely lacking for developing countries [10,60,63]. HPV positive tumors are typically diagnosed at higher stages but are more responsive to treatment, providing important implications for screening and quality of care [64,65]. While pharyngeal cancer rates appear to be decreasing among both genders in Lampang, Chiang Mai, Singapore, the US as well as Filipino females, tongue cancer incidence rates appear to be much

more stagnant, with significant decreases localized to Filipino and Chiang Mai females. Although etiological inference is limited, further research is needed to assess the potential role of HPV in HNSCC within Asian populations.

This is the first study, to our knowledge, to assess temporal trends in overall and site specific HNSCC across southeast and eastern Asia in comparison to the US. The use of high-quality IARC CI5 cancer incidence data is a strength in our study, however, there are also several limitations. Data ascertainment is limited by geographical coverage and quality, subsequently leading to restrictions on generalizability to country-wide incidence rates. Stratification by nationality, or sub-population (i.e. patients coming from urban vs. rural settings) were also either limited by sample size or availability of data, restricting interpretability. Finally, inception years, conversion to population-based systems, and variability in data sources and follow-up methods between registries limit comparability [66].

In general, HNSCC incidence appears to be decreasing across parts of Asia and the US, with the exception of Shanghai males. While introduction of tobacco control policies in places such as Thailand and Singapore have led to decreases in risk behaviors and subsequent cancer incidence, concerns regarding China's continuing wide-scale use of tobacco products likely plays a role in escalating laryngeal cancer rates within this population and should be closely monitored. Additionally, further studies should be conducted to evaluate the potential role of HPV in oral and oropharyngeal cancer, particularly in economically transitioning countries, where adaptation of western cultures may reflect on cancer incidence rates.

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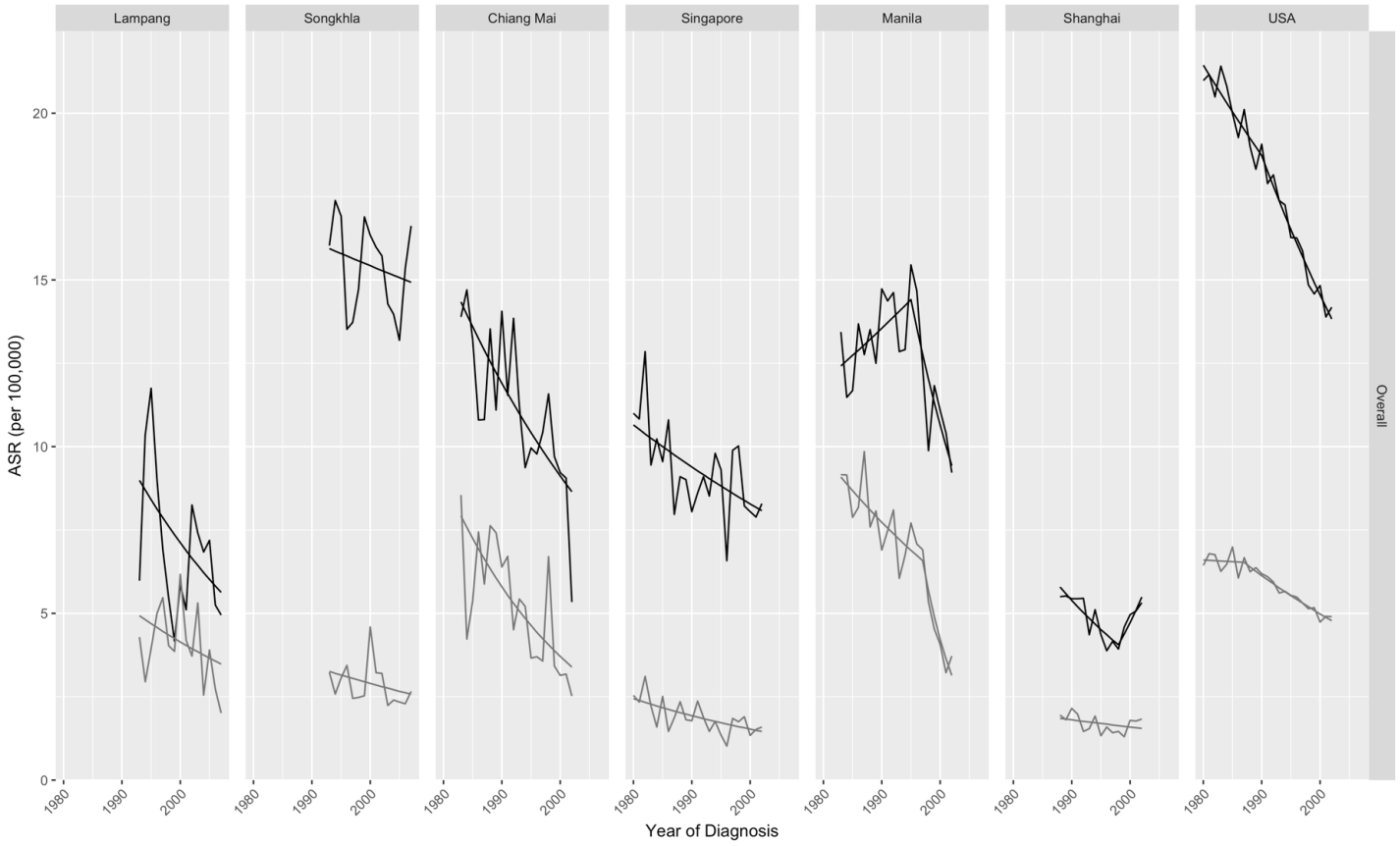
Table 4.1: HNSCC case distribution by registry and sex

	Lampang		Songkhla		Chiang Mai		Singapore		Manila		Shanghai		United States	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Diagnosis Period	1993-2007		1993-2007		1983-2002		1980-2007		1983-2002		1988-2002		1980-2002	
Number of Cases , N (%)	453 (61.22)	287 (38.78)	1367 (81.51)	310 (18.49)	1396 (67.96)	658 (32.04)	3754 (80.80)	892 (19.20)	2777 (60.81)	1790 (39.19)	3439 (71.65)	1361 (28.35)	53980 (72.28)	20698 (27.72)
Age at diagnosis, N (%)														
≤50	81 (17.88)	45 (15.68)	177 (12.95)	29 (9.35)	163 (11.68)	99 (15.05)	457 (12.17)	197 (22.09)	544 (19.59)	301 (16.82)	406 (11.81)	209 (15.36)	7105 (13.16)	2571 (12.42)
51-60	65 (14.35)	39 (13.59)	234 (17.12)	81 (26.13)	248 (17.77)	142 (21.58)	880 (23.44)	169 (18.95)	766 (27.58)	377 (21.06)	548 (15.93)	189 (13.89)	13008 (24.10)	4182 (20.20)
61-70	123 (27.15)	86 (29.97)	423 (30.94)	41 (13.23)	502 (35.96)	221 (33.59)	1270 (33.83)	245 (27.47)	880 (31.69)	540 (30.17)	1143 (33.24)	411 (30.20)	17273 (32.00)	6034 (29.15)
71-80	127 (28.04)	78 (27.18)	533 (38.99)	159 (51.29)	483 (34.60)	196 (29.79)	948 (25.25)	209 (23.43)	587 (21.14)	572 (31.96)	1057 (30.74)	392 (28.80)	12218 (22.63)	5051 (24.40)
≥81	57 (12.58)	39 (13.59)					199 (5.30)	72 (8.07)			285 (8.29)	160 (11.76)	4376 (8.11)	2860 (13.82)
Year of diagnosis, N (%)														
1980-1983					63 (4.51)	36 (5.47)	457 (12.17)	121 (13.57)	113 (4.07)	92 (5.14)			9587 (17.76)	3445 (16.64)
1984-1987					254 (18.19)	120 (18.24)	454 (12.09)	99 (11.10)	461 (16.60)	369 (20.61)			9556 (17.70)	3593 (17.36)
1988-1991					288 (38.83)	165 (25.08)	466 (12.41)	129 (14.46)	552 (19.88)	363 (20.28)	993 (28.87)	407 (29.90)	9365 (17.35)	3646 (17.62)
1992-1995	104 (22.96)	44 (15.33)	238 (17.41)	53 (17.15)	291 (20.85)	131 (19.91)	531 (14.14)	115 (12.89)	615 (22.15)	393 (21.96)	935 (27.19)	334 (24.54)	9416 (17.44)	3671 (17.74)
1996-1999	103 (22.74)	85 (29.62)	327 (23.92)	74 (23.95)	316 (22.64)	129 (19.60)	598 (15.93)	132 (14.80)	607 (21.86)	380 (21.23)	793 (23.06)	318 (23.37)	9142 (16.94)	3642 (17.60)
2000-2003	123 (27.15)	97 (33.80)	385 (28.16)	96 (31.07)	184 (13.18)	77 (11.70)	584 (15.56)	143 (16.03)	429 (15.45)	193 (10.78)	718 (20.88)	302 (22.19)	6914 (12.81)	2701 (13.05)
2004-2007	123 (27.15)	61 (21.25)	417 (30.50)	86 (27.83)			664 (17.69)	153 (17.15)						
Sub-Site, N (%)														
Oral Cavity	108 (23.84)	140 (48.78)	340 (24.87)	173 (55.99)	294 (21.06)	234 (35.56)	615 (16.38)	277 (31.05)	587 (21.14)	680 (37.99)	640 (18.61)	568 (41.73)	15065 (27.91)	7401 (35.76)
Tongue	87 (19.21)	54 (18.82)	319 (23.34)	63 (20.39)	210 (15.04)	118 (17.93)	587 (15.64)	283 (31.73)	484 (17.43)	450 (25.14)	418 (12.15)	385 (28.29)	8521 (15.79)	4501 (21.75)
Larynx	153 (33.77)	56 (19.51)	291 (21.29)	25 (8.09)	489 (35.03)	164 (24.92)	1710 (45.55)	181 (20.29)	1225 (44.11)	265 (14.80)	2066 (60.08)	270 (19.84)	19094 (35.37)	4679 (22.61)
Pharynx	105 (23.18)	37 (12.89)	417 (30.50)	48 (15.53)	403 (28.87)	142 (21.58)	842 (22.43)	151 (16.93)	481 (17.32)	395 (22.07)	315 (9.16)	138 (10.14)	11300 (20.93)	4117 (19.89)

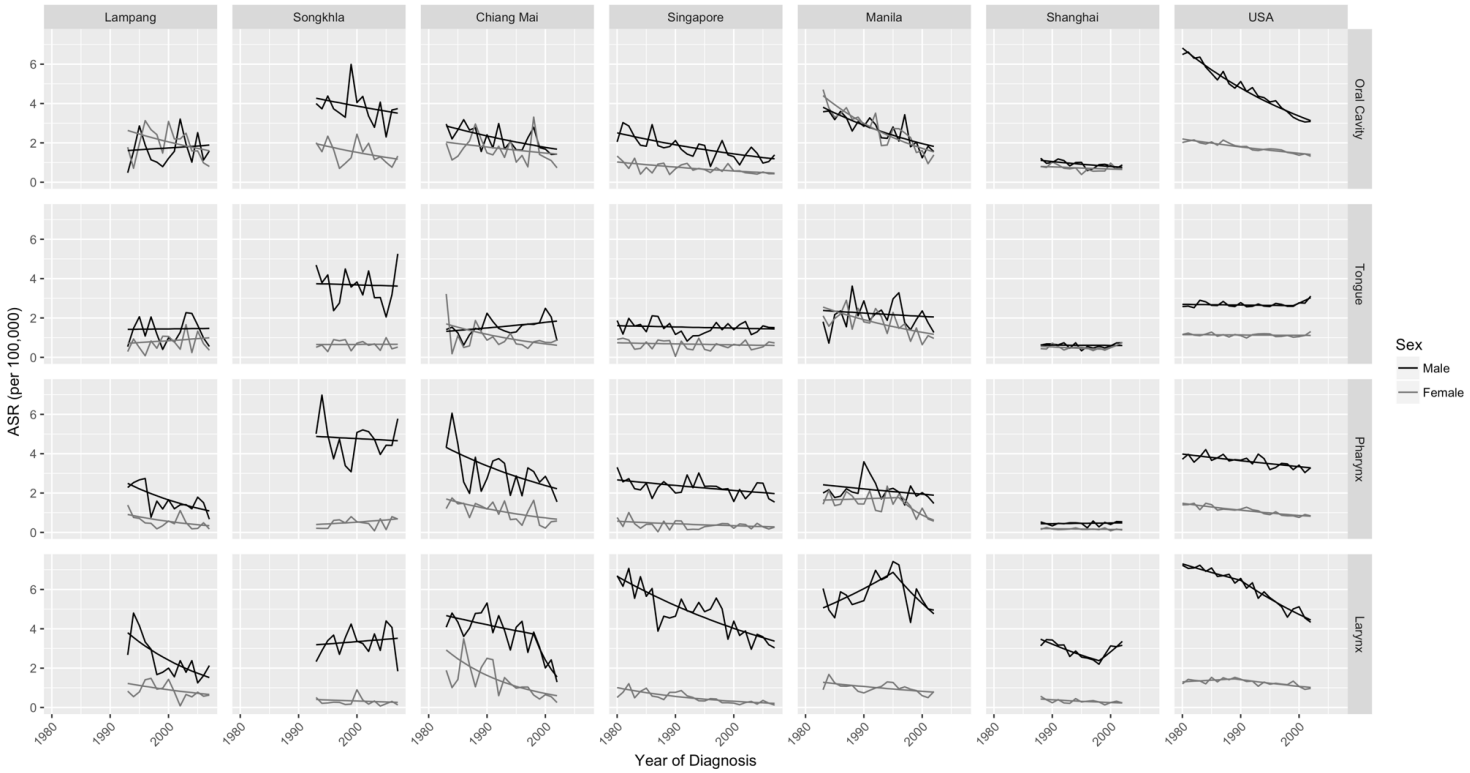
Figure 4.1: Joinpoint analyses of temporal trends in overall HNSCC by sex and registry APC (95% CI**)**



	Lampang	Songkhla	Chiang Mai	Singapore	Manila	Shanghai	USA
Overall Head and Neck							
Males	**-3.3* (-6.5, -0.0)**	-0.5 (-1.6, 0.7)	**-2.5* (-3.8, -1.2)**	**-1.9* (-2.3, -1.5)**	1.2 (-0.3, 2.8) **-5.9* (-8.9, -2.8)**	**-3.5* (-5.1, -1.9)** **7.9* (1.1, 15.1)**	**-1.3* (-1.9, -0.8)** **-2.5* (-2.9, -2.1)**
Females	-2.5 (-6.2, 1.5)	-1.7 (-4.4, 1.2)	**-4.5* (-6.6, -2.4)**	**-2.7* (-3.4, -1.9)**	**-2.3* (-3.6, -1.0)** **-13.8* (-19.9, -7.1)**	-1.3 (-3.1, 0.6)	-0.2 (-1.5, 1.2) **-2.1* (-2.5, -1.6)**

* Denotes statistical significance at α=0.05

Figure 4.2: Joinpoint analyses of temporal trends in subsite specific HNSCC by sex and registry APC (95%CI)



	Lampang		Songkhla		Chiang Mai		Singapore		Manila		Shanghai		USA	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Oral Cavity	1.1 (-5.1, 7.7)	-3.5 (-8.6, 1.9)	-1.4 (-4.2, 1.5)	-3.6 (-7.8, 0.7)	**-2.8* (-4.5, -1.0)**	-1.9 (-5.3, 1.7)	**-2.7* (-3.8, -1.7)**	**-2.9* (-4.2, -1.7)**	**-3.8* (-5.3, -2.4)**	**-5.4* (-6.9, -3.8)**	**-2.7* (-4.6, -0.7)**	-1.5 (-4.3, 1.3)	**-3.5* (-3.8,-3.2)**	**-2.0* (-2.3, -1.7)**
Tongue	0.2 (-6.2, 7.1)	2.4 (-5.9, 11.3)	-0.2 (-3.5, 3.1)	0.0 (-5.1, 5.4)	1.8 (-0.6, 4.1)	**-5.2* (-8.7, -1.5)**	-0.4 (-1.4, 0.6)	-0.8 (-2.4, 0.9)	-0.8 (-3.5, 2.0)	**-4.0* (-6.4, -1.6)**	-0.2 (-2.8, 2.5)	-2.8 (-7.0, 1.7) 22.5 (-6.7, 61.0)	-0.1 (-0.5, 0.2) 4.9 (-0.6, 10.6)	-0.2 (-0.6, 0.3)
Pharynx	**-5.7* (-9.2, -2.1)**	**-6.8* (-13.0, -0.1)**	-0.3 (-2.9, 2.3)	3.9 (-2.5, 10.8)	**-3.4* (-5.6, -1.3)**	**-4.8* (-8.2, -1.3)**	**-1.1* (-1.9, -0.3)**	**-2.5* (-4.7, -0.4)**	-1.3 (-3.2, 0.6)	0.6 (-3.6, 4.9) **-16.0* (-27.9, -2.1)**	0.7 (-2.3, 3.7)	-1.9 (-5.3, 1.6)	**-0.9* (-1.3, -0.5)**	**-2.7* (-3.2, -2.1)**
Larynx	**-6.3* (-9.5, -3.0)**	-4.3 (-9.8, 1.6)	0.7 (-2.4, 3.9)	-3.1 (-11.8, 6.4)	-1.5 (-3.7, 0.8) **-19.7* (-34.2, -2.0)**	**-8.0* (-11.7, -4.2)**	**-2.5* (-3.1, -1.9)**	**-5.6* (-7.2, -4.1)**	**2.6* (0.4, 4.8) -5.1* (-9.1, -0.9)**	**-2.6* (-4.4, -0.7)**	**-3.7* (-5.3, -2.1) 9.1* (2.2, 16.4)**	**-4.3* (-7.9, -0.7)**	**-1.2* (-2.1, -0.4) -3.1* (-3.7, -2.4)**	1.2 (-1.2, 3.7) **-2.7* (-4.1, -1.3)**

* Denotes statistical significance at α=0.05

Chapter 5

Temporal Changes in Head and Neck Cancer Incidence in Thailand Suggest Changing Oropharyngeal Epidemiology in the Region

Abstract

Background: Head and neck cancer (HNC) is the sixth most common cancer in the world, with the largest burden occurring in developing countries. Although the primary risk factors have been well characterized, little is known about temporal trends in HNC across Thailand.

Methods: Head and neck squamous cell carcinoma (HNSCC) cases diagnosed between 1990 and 2014 were selected by ICD-10 code from the Songkhla, Lampang, Chiang Mai and Khon Kaen Cancer Registries and the U.S. Surveillance, Epidemiology, and End Results Program for: oral cavity (00, 03-06), tongue (01-02), pharynx (09-10, 12-14), and larynx (32). The data were analyzed using R and Joinpoint Regression Software to determine age-standardized incidence rates and trends of annual percent change (APC). Incidence rates were standardized using the Segi (1960). Stratified linear regression models were conducted to assess temporal trends in early onset HNSCC across 20-year age groups.

Results: While overall HNSCC rates are decreasing across all registries, subsite analyses demonstrate consistent decreases in both larynx and oral cavity cancers, but suggested increases in tongue cancers among both sexes in the US (APC_M: 2.36 and APC_F: 0.77) as well as pharyngeal cancer in Khon Kaen and US males (APC: 2.1 and APC: 2.23, respectively). Age-stratified APC analyses assessing young onset (<60 years old) trends demonstrated increased incidence in tongue cancer in Thailand and the U.S., as well as pharyngeal cancers in Khon Kaen males age 40-59 and US males age 50-59.

Conclusions: Although overall trends in HNSCC are decreasing across both Thailand and the United States, there is reason to believe that the etiological shift to oropharyngeal cancers in the US may be occurring in Thailand.

Introduction:

Worldwide, head and neck cancer (HNC) accounts for more than 550,000 cases and 300,000 deaths each year [1,2], with a dismal five-year overall survival rate of 40-50% [3]. HNC is the sixth most common cancer in the world [4], with the highest incidence observed in South and Southeast Asia [5,6]. Many of these cases are attributed to use of betel quid, although there have been no systematic studies of risk factors for this set of cancers within these regions of the world. In the western world, decreased combustible tobacco use and increased human papillomavirus (HPV) prevalence [7] have allowed for an epidemiological shift that has highlighted the role of high-risk HPV strains in head and neck squamous cell carcinoma (HNSCC) pathology [8]. This subset of the disease is seen more often in younger patients, and is most commonly presents in the oral cavity, tongue and oropharynx [9,10]. However, most of these data are from Western countries, with little knowledge regarding the presentation and prevalence of this important etiological factor in other high incidence areas such as Thailand.

A first step to addressing the burden of HNC in Southeast Asia is to analyze surveillance data for trends in incidence of HNSCC. In the past few decades, rapid socioeconomic development has allowed for improved control of communicable diseases, resulting in the emergence of cancer as the leading cause of death [11]. Thailand has an efficient universal healthcare system that, among many other benefits, facilitates the collection of health statistics [12]. Subsequently, Thailand's high quality cancer statistics are obtained and managed by 16 province- and regional- based registries that build on the healthcare infrastructure [13]. Additionally, the sociocultural factors in Thailand are similar to those in surrounding countries, and thus, incidence trends may reflect regional changes in HNSCC.

Here we take advantage of data available from the Thai Cancer Information Network to evaluate incidence trends in four areas of Thailand. We identify evidence for increases in HPV-associated oropharyngeal cancer similar to those seen in the United States (U.S.), while noting overall decreases in historically smoking and alcohol associated cancers across all study sites.

Methods:

HNSCC incidence data available from 1990-2014 were obtained from the Songkhla, Khon Kaen, Lampang and Chiang Mai cancer registries, and compared to those found in the U.S. Surveillance, Epidemiology, and End Results Program (SEER) (**Figure 5.1**) [14-18]. Each Thai registry has case ascertainment of ~ 90% within their catchment area [19]. Chiang Mai, which was the first cancer registry to be established in Thailand, has actively obtained cancer cases from all provincial hospitals within the province since 1983 [20]. Lampang passively collects data from cancer centers and all public and private hospitals within the province [21]. Khon Kaen, which represents an estimated third of the overall population of Thailand, collects data from all care facilities using both active and passive methods [22]. The province of Songkhla has a population-based registry that was established in 1989 and managed by the Prince of Songkla University [23]. Finally, the U.S. SEER database which was established in 1973 and uses both passive and active data collection (**Table 5.3**). All registries provide data for the International Agency for Research on Cancer's (IARC) Cancer Incidence in Five Continents repository (CI5)[24].

Inclusion criteria for case selection was based upon International Classification of Disease, 10th Edition (ICD-10) codes. Site-specific cancers were defined by the following codes: oral cavity (C00, C03-C06), tongue (C01-C02), pharynx (C09-C10, C12-C14) and larynx (C32). Overall HNSCC incidence was calculated as the sum across all subsites. In addition to diagnosis, case information included: age, sex, date of diagnosis, stage, histology and morphology. For the Thai data, population denominators by registry, year, age and sex were based upon decennial census data from 1990, 2000, and 2010, which were conducted by the Thai National Statistical Office [25,26]. Annual intercensal population structures for the various provinces were estimated by 5-year sex-specific age groups, using a log-linear function between successive censuses. Population counts beyond 2010 were estimated by the Office of the National Economic and Social Development Board [27,28]. Population denominators for the SEER data were obtained from SEER*Stat 8.3.5[18] and based upon the US Census Bureau's Population Estimates Program[29].

Data were analyzed using R-statistical software version 3.3.3 [30]. To assess sex-specific HNSCC trends, annual percent change (APC) was calculated using age-adjusted incidence rates in

Joinpoint regression model version 4.5.0.1. [31]. This software utilizes a Monte Carlo permutation method to assess the number of joinpoints, slope in the trends and their significance [32]. When no cases were present within a given year, a half-case was added to the age strata with the largest population to enable computation on the log-linear scale [14,32]. This method was repeated for all sub-sites, standardized to the Segi (1960) world population for comparability [33,34]. Additional exploratory analyses using stratified linear regression models were conducted to assess temporal trends in earlier onset HNSCC across 20-year age grouping.

Results

Between 1990 and 2014, 91,491 cases of HNSCC were reported in the U.S., 2,938 in Chiang Mai, 2,158 in Khon Kaen, 2,947 in Songkhla, and 1,202 in Lampang. The distribution of age at diagnosis was relatively similar across all study sites, with means ranging from 61-67 years old. HNSCC incidence was higher among males than females in all registries except Khon Kaen, where females represented 50.14% of the observed cancer cases. In the U.S., Chiang Mai and Lampang, laryngeal cancer accounted for the highest proportion of HNSCC among men, while oral cavity and pharynx cancers were predominant in Khon Kaen and Songkhla, respectively. Among females, oral cavity cancers contributed the greatest case burden across all registries (**Table 5.1**).

Songkhla was found to have the highest overall HNSCC incidence among men, with an expected age standardized rate (EASR) of 14.68 per 100,000 in 2014. Although much lower among males, the U.S. stood out as having the highest HNSCC burden among females, with an $EASR_{2014}$ of 4.32 per 100,000. Overall, these rates are decreasing across all registries except Khon Kaen and Chiang Mai, where there is an observed, non-statistically significant increase in incidence among males (APC: 0.5 [95%CI: -0.6, 1.6] and APC: 8.1 [95%CI: -3.3, 20.9] respectively). Despite these general decreases, analyses assessing sub-site incidence trends demonstrated great variability in slope, and corresponding significance, across registries and anatomical location (**Figure 5.2**).

When compared to other registries, laryngeal cancer rates were highest among US women and men ($EASR_{1990\text{-}2014}$: 1.0 and 4.7 per 100,000, respectively). Laryngeal cancer among women is significantly decreasing in Lampang (APC: -4.8 [95%CI: -7.7, -1.9]), Chiang Mai (APC: -11.2 [95%CI: -13.7, -8.7]), and the U.S. (APC: -2.7 [95%CI: -3.2, -2.3]). Among men, these trends have been more variable, with the most recent significant decreases observed in Lampang from 2007-2014 (APC: -11.8 [95%CI: -19.3, -3.5]), and the U.S. (APC: -2.8 [95%CI: -3.0, -2.5]) (**Figure 5.2**).

Khon Kaen women and Songkhla men were found to have to highest oral cavity cancer rates, both with $EASR_{1990\text{-}2014}$ of 3.8 per 100,000. Although oral cavity cancers contribute the greatest proportion of HNSCC cases among females, the incidence of these cancers has declined across all registries, with significant decreases observed in Songkhla (APC: -5.1 [95%CI: -6.8, -3.4]), Khon Kaen (APC: -4.6 [95%CI: -6.0, -3.1]), Lampang (APC: -3.1 [95%CI: -5.4, -0.8]) and the U.S. (APC: -1.8 [95%CI: -2.1, -1.5]). Similarly, trends among men also note reductions in oral cavity cancer incidence, particularly in Songkhla (APC: -2.1 [95%CI: -3.4, -0.8]), Khon Kaen (APC: -2.6 [95%CI: -4.5, -0.7]) and Lampang (APC: -3.1 [95%CI: -5.8, -0.3]) (**Figure 5.2**).

Pharyngeal cancer rates were highest among US females ($EASR_{1990\text{-}2014}$: 1.0 per 100,000) and Songkhla males ($EASR_{1990\text{-}2014}$: 5.1 per 100,000). Decreased incidence rates were noted among Chiang Mai females (APC: -8.2 [95%CI: -9.9, -6.4]) as well as both females and males in Lampang (APC: -4.0 [95%CI: -7.0, -0.9]; APC: -4.5 [95%CI: -6.1, -2.8], respectably). Conversely, increases in pharyngeal cancer incidence were found among males in Khon Kaen (APC: 2.1 [95%CI: 0.4, 3.8]) and the U.S. from 2002-2014 (APC: 2.2 [95%CI: 1.5, 3.0]). Tongue cancer rates were highest among US females ($EASR_{1990\text{-}2014}$: 1.2 per 100,000) and Songkhla males ($EASR_{1990\text{-}2014}$: 3.9 per 100,000). These rates were found to be significantly increasing among females in the U.S. (APC: 0.8 [95%CI: 0.4, 1.2]) as well as males from 1998-2014 (APC: 2.4 [95%CI: 1.9, 2.9]). A suggested increase in incidence of tongue cancer was observed among Lampang males, as well as both sexes in Khon Kaen and Chiang Mai, but these trends did not reach statistical significance (**Figure 5.2**).

Exploratory analyses into younger onset (< 60 years old) trends demonstrated significant increases in tongue cancer among Chiang Mai females age 50-59, Khon Kaen females age 30-39, Khon Kaen males age 30-59, Lampang males age 50-59, Songkhla females age 30-49, U.S. females 30-49 as well as US males age 40-59. Pharyngeal cancers were also seen to increase in Khon Kaen males age 40-59, and U.S. males age 50-59 (**Table 5.2**) (**Figure 5.2**).

Discussion

In this study, we found HNSCC decreasing across four registries in Thailand, as well as the U.S., with distinct variability across anatomical sub-site, sex and geographical location. While the vast majority of these changes may be attributed to anti-smoking campaigns, changes in policy and consequent shifts in risk behaviors, there also appears to be evidence suggesting the growing impact of HPV-related etiology on increasing oropharyngeal cancer incidence in Thailand and the U.S.

HNC is comprised of highly heterogeneous cancers, with global incidence reflective of trends in tobacco and alcohol use [35,36], and sexual norms [37,38]. As these risk behaviors change over time, the site-specific incidence contributing to overall HNC trends reflect these changes in a population- and gender-specific manner. In 1990, the largest proportion of HNSCC among men were observed in the larynx in the U.S. and Lampang ($EASR_{1990}$ of 6.40 and 1.89 per 100,000, respectively), oral cavity in Khon Kaen and Songkhla ($EASR_{1990}$: 2.09 and 4.97 per 100,000, respectively), and pharynx in Chiang Mai ($EASR_{1990}$: 6.67 per 100,000). In contrast, in 2014 the largest drivers of HNSCC among men shifted to pharyngeal cancer in the U.S., Songkhla and Chiang Mai ($EASR_{2014}$: 4.54, 4.70 and 2.33 per 100,000, respectively) and tongue cancer in Khon Kaen and Lampang ($EASR_{2014}$: 1.62 and 1.83 per 100,000, respectively). Among females, oral cavity cancers appear to have historically predominated HNSCC incidence across all registries and, although to a lesser degree, continue to do so in Khon Kaen, Chiang Mai and Lampang; on the other hand, in the U.S. and Songkhla, these trends appear to have shifted in recent years, resulting from a decrease in oral cavity incidence and an increase in tongue cancer. Nevertheless, HNSCCs among women have significantly decreased across all Thai registries, while such observations are less apparent among males.

While an estimated 7% of oral cavity cancers in the U.S. are attributed to chewing tobacco [39], the practice of chewing betel quid, comprised of areca-nut, slaked lime, betel leaf and often tobacco, is widely prevalent throughout Thailand. A study conducted in Northeastern Thailand found that ever chewers of betel quid had a 9.01-fold increased risk of oral cancer compared to never chewers in multivariate analyses [40]; numerous other studies have also implicated betel quid use in the etiology of oral cavity cancer [40-45]. Several studies from northeastern Thailand have demonstrated the high prevalence of betel use among women, commonly used as a social activity, particularly among older generations [42,45,46]. Conversely, chewing tobacco use among women in the US has historically been low; according to the National Survey on Drug Use and Health, an estimated 0.5% of adult women utilized smokeless tobacco in the U.S. in 2016[47]. After years of education and political campaigns to decrease betel-quid use in Thailand, recent data shows declines in this risk behavior, especially among younger populations [13,42]. The impact of this cultural shift is apparent in the consistent decreases in oral cavity cancers, particularly among women, and most notably in Khon Kaen, where the incidence reduced by two-thirds: from an $EASR_{1990}$ of 6.37 per 100,000 to an $EASR_{2014}$ of 2.08 per 100,000 (APC: -4.6 [95%CI: -6.0, -3.1]).

Tobacco use has long been considered the leading cause of HNSCC, often working synergistically with alcohol consumption [48,49]. A study in the International Head and Neck Cancer Epidemiology Consortium estimated that ~90% of HNSCC patients have a history of tobacco use [50], conferring a 10-fold increase in laryngeal cancer risk and a 4 to 5-fold increase in hypopharynx, oropharynx and oral cavity cancer risk [51]. Being dose-dependent in nature [50,52], several studies have suggested smoking cessation to reduce the risk of HNSCC [53,54]. In the U.S., increased societal awareness and support for combustible tobacco control has led to a decrease in cigarette consumption [55,56], and consequently laryngeal cancer rates, with an APC of -2.8% (95%CI: -3.0, -2.5) among men and -2.7% (95%CI: -3.2, -2.3) among females. Beginning in 1991, Thailand was the first nation in Asia to implement strong tobacco control policies [57,58]. Since then, numerous regulations have been put into place, including bans on advertisement and smoking in certain public places, health warnings, and taxation [59,60]. As a result of these aggressive control methods, the overall smoking rate in Thailand has dropped from 30% in 1976 to 19.9% in 2013 [61] and lung cancer rates, particularly in Northern Thailand, have also decreased

[62]. As demonstrated in **Figure 5.2**, EASR of laryngeal cancer among females in Thailand has been, and remains, relatively low, with observed decreases across all registries. In addition to the aforementioned control methods, cultural norms in Thailand have historically resulted in women abstaining from smoking and drinking [13,61]. Interestingly, comparing reported alcohol consumption rates in Thailand between 2003/2004 to those in 2008/2009 demonstrated decreased drinking rates among young men and increasing rates among young women, pointing to a potential change in cultural practices [63,64]. Although inconsistent across time, significant decreases in laryngeal cancer incidence can be observed among men in Lampang, with an APC -11.8% (95%CI: -19.3, -3.5) from 2007-2014. Chiang Mai also shows decreased incidence of laryngeal cancer among men, but these trends fail to reach statistical significance, while trends in both Khon Kaen and Songkhla remain stagnant.

Historically, tongue and pharyngeal tumors have been associated with similar risk factors as other HNSCC sites, such as combustible tobacco and alcohol [52,65]. An etiological shift in oropharyngeal tumors in the U.S. has led to increased global awareness of high-risk HPV as a causal factor in carcinogenesis of these sites, with current prevalence estimates in the U.S. near 80% [8,66-72]. While pharyngeal cancer incidence rates varied widely across the different registries, tongue cancer rates appeared to increase across all registries except both genders in Songkhla and Lampang females. Studies assessing the differences in HPV-positive and negative HNSCC demonstrated an increase in HPV-positive tumors among younger cohorts with a lower smoking prevalence and multiple sexual partners [72]. In an exploratory analysis to assess the change in incidence of tongue and pharynx cancer among younger individuals (<60 years old) we found significant increases in tongue cancer across all registries, as well as pharyngeal cancer in Khon Kaen males (**Table 5.2**). A sensitivity analysis to assess these trends in the oropharynx as defined by ICD-10 codes: C01, C02.4, C09.0-09.9, and C10.0-10.9 [8] demonstrated significant increases in incidence rates among Khon Kaen males age 30-39 and 50-59, Khon Kaen females age 40-49, Songkhla females age 50-59 and Lampang females age 30-39 (data not shown). To date, there have only been two studies conducted in the Thai population to assess HPV prevalence in HNSCC—both in the oral cavity [73,74]. The more recent of the two studies utilized 146 archival tissue samples of oral squamous cell carcinoma, and found 56.2% to be positive for high risk HPV, and 43.8% to express HPV E6/E7 mRNA [73].

This study was the first, to our knowledge, to assess HNSCC trends across multiple registries in Thailand in comparison to the U.S. While data obtained from population-based registries allow for results to be extrapolated to the pertinent provinces, it is difficult to approximate the number of unreported cases of cancer, particularly among those living in rural villages. The introduction of universal health coverage by the Thai National Health Security Office in 2002 allowed for improved access to care, leading to a presumed increase in case ascertainment and, consequently, increased incidence trends. In this study, however, we did not observe any significant changes in incidence between the periods before and after the introduction of this policy, noting primarily decreases in overall HNSCC incidence.

The comparison of trends across different registries poses challenges not only due to variability in data collection methods, but also in population composition. While Thailand's registries boast high quality data collection [16,19], with methods varying from passive, to active, to a combination of the two [20-23], analyses of incidence near the inception of a given registry may lead to concerns regarding data reliability; this is particularly relevant in Lampang, where registry data prior to 1993 was collected retrospectively, and may be biased towards cases alive at the time of the registry's foundation. Nevertheless, this study assessed HNSCC trends across twenty-four years, resulting in a comprehensive temporal analysis. Finally, changes in population structure have become increasingly apparent across Thailand, with rural to urban migration of individuals within Thailand, as well as immigration into the country [75,76].

In conclusion, although HNSCC appears to be decreasing across Thailand as well as the U.S., there is evidence to suggest that the increase in high-risk HPV associated oropharyngeal cancers previously reported in the U.S., may also be relevant in Thailand. HPV positive tumors have been shown to be more responsive to treatment, ultimately leading to suggested restaging, and consequently less aggressive treatment, of these lesions by the American Joint Committee on Cancer [77]. Due to the younger onset nature of these lesions, de-escalation of treatment may lead to improvements in morbidity and mortality of afflicted patients. With the potential increase in HPV-positive tumors in Thailand, further molecular studies are necessary in order to evaluate

oropharyngeal etiology, subsequently leading to improved public health practice and treatment protocols across Thailand.

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Table 5.1: Head and Neck Cancer Distribution, 1990-2014, by Region, Sex and Site

	USA		Chiang Mai		Lampang		Khon Kaen		Songhkla	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Mean Age, y	62.63	65.29	64.72	65.64	63.73	65.99	61.05	66.98	64.42	67.25
Number of Cases, N (%)	66,087 (72.23)	25,404 (27.77)	2,008 (68.35)	930 (31.65)	732 (60.90)	470 (39.10)	1,076 (49.86)	1,082 (50.14)	2,407 (81.68)	540 (18.32)
Subsite, N (%)										
Oral Cavity	15,353 (23.23)	8,744 (34.42)	463 (23.06)	432 (46.45)	163 (22.27)	214 (45.53)	286 (26.58)	799 (73.84)	559 (23.22)	273 (50.56)
Tongue	14,142 (21.40)	6,451 (25.39)	367 (18.28)	166 (17.85)	164 (22.40)	97 (20.64)	266 (24.72)	202 (18.67)	573 (23.81)	138 (25.56)
Pharynx	16,801 (25.42)	5,205 (20.49)	585 (29.13)	182 (19.57)	170 (23.22)	72 (15.32)	249 (23.14)	58 (5.36)	759 (31.53)	87 (16.11)
Larynx	19,791 (29.95)	5,004 (19.70)	593 (29.53)	150 (16.13)	235 (32.10)	87 (18.51)	275 (25.26)	23 (2.13)	516 (21.44)	42 (7.78)

Table 5.2: Sex and Age Group Stratified Young Onset Cancer Rates by Site and Registry [APC (p-value)]

	USA		Chiang Mai		Lampang		Khon Kaen		Songhkla	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
H&N										
30-39	**-0.129 (<0.0001)**	0.012 (0.08)	-0.022 (0.64)	**-0.097 (<0.0001)**	0.033 (0.34)	0.037 (0.41)	0.067 (0.08)	0.013 (0.72)	**0.106 (0.01)**	**0.054 (0.03)**
40-49	**-0.200 (<0.0001)**	-0.013 (0.37)	-0.098 (0.37)	**-0.127 (0.01)**	-0.023 (0.80)	0.016 (0.77)	**0.264 (0.001)**	-0.013 (0.74)	0.149 (0.18)	0.055 (0.09)
50-59	**-0.302 (0.003)**	**-0.209 (<0.0001)**	**-0.515 (0.01)**	**-0.644 (<0.0001)**	-0.048 (0.79)	**-0.261 (0.02)**	**0.275 (0.02)**	**-0.372 (0.002)**	0.337 (0.11)	-0.159 (0.16)
Oral Cavity										
30-39	**-0.095 (<0.0001)**	-0.003 (0.37)	0.015 (0.48)	**-0.057 (0.001)**	-0.023 (0.27)	0.001 (0.98)	-0.013 (0.18)	**-0.039 (0.02)**	0.013 (.55)	0.012 (0.26)
40-49	**-0.158 (<0.0001)**	**-0.014 (0.02)**	-0.055 (0.20)	-0.028 (0.03)	0.016 (0.64)	0.022 (0.75)	0.009 (0.77)	-0.029 (0.28)	0.003 (0.96)	0.001 (0.98)
50-59	**-0.355 (<0.0001)**	**-0.098 (<0.0001)**	**-0.092 (0.04)**	**-0.270 (0.02)**	-0.004 (0.95)	0.059 (0.25)	-0.048 (0.45)	**-0.375 (<0.0001)**	0.035 (0.74)	-0.071 (0.22)
Tongue										
30-39	-0.002 (0.77)	**0.017 (0.001)**	0.006 (0.78)	-0.020 (0.19)	0.024 (0.24)	0.024 (0.35)	**0.053 (0.02)**	**0.051 (0.01)**	0.036 (0.12)	**0.038 (0.02)**
40-49	**0.028 (0.04)**	**0.021 (0.01)**	-0.042 (0.28)	-0.028 (0.25)	0.063 (0.24)	0.022 (0.58)	**0.118 (0.003)**	0.021 (0.28)	0.086 (0.28)	**0.050 (0.04)**
50-59	**0.218 (<0.0001)**	0.019 (0.13)	-0.013 (0.86)	**0.067 (0.04)**	**0.222 (0.001)**	-0.0001 (0.99)	**0.098 (0.04)**	0.016 (0.73)	-0.035 (0.67)	-0.048 (0.34)
Pharynx										
30-39	**-0.017 (0.01)**	0.004 (0.21)	-0.034 (0.22)	-0.010 (0.09)	0.032 (0.11)	0.008 (0.76)	0.027 (0.11)	-0.008 (0.49)	0.037 (0.16)	0.004 (0.77)
40-49	0.032 (0.14)	-0.004 (0.44)	-0.018 (0.61)	-0.036 (0.10)	-0.043 (0.27)	-0.014 (0.46)	**0.081 (0.03)**	0.016 (0.30)	0.086 (0.14)	0.011 (0.38)
50-59	**0.203 (<0.0001)**	**-0.041 (0.03)**	-0.059 (0.40)	**-0.183 (0.0004)**	-0.059 (0.54)	**-0.078 (0.02)**	**0.151 (0.01)**	-0.006 (0.74)	0.135 (0.34)	-0.027 (0.48)
Larynx										
30-39	**-0.014 (0.001)**	**-0.006 (0.04)**	-0.009 (0.19)	-0.010 (0.09)	-0.001 (0.96)	-0.001 (0.89)	-0.001 (0.95)	0.009 (0.10)	0.020 (0.24)	--
40-49	**-0.102 (<0.0001)**	**-0.016 (0.01)**	0.018 (0.65)	**-0.035 (0.01)**	-0.059 (0.19)	0.002 (0.92)	0.056 (0.09)	-0.015 (0.25)	-0.026 (0.48)	-0.006 (0.59)
50-59	**-0.367 (<0.0001)**	**-0.088 (<0.0001)**	**-0.351 (0.001)**	**-0.259 (0.0003)**	**-0.207 (0.01)**	**-0.112 (0.02)**	0.074 (0.16)	-0.007 (0.47)	0.202 (0.06)	-0.012 (0.69)

Table 5.3: Registry Characteristics and Data Quality Measures

	USA (SEER-9)	Chiang Mai	Lampang	Khon Kaen	Songhkla
Year Started	1973	1978	1993	1985	1988
Years Data Collected Retrospectively	N/A	N/A	1990-1992	N/A	N/A
Active/Passive	Active/Passive	Active	Passive	Active/Passive	Active
Population Based (Y/N)- Year Started*	Y-1973	Y-1983	Y-1995	Y-1985	Y-1989
Data Sources*◊	A-M, O	A-H, J, L-M, P	A-H, J, L-M, P	A-J, L-N, P	A-H, J, L-M
Population in Urban Area (%)*	N/A	24	30	21	38
% Morphologically Verified for Males, Females*					
Oral cavity/pharynx (C00-14)	98.7, 98.1	92.9, 95.0	84.9, 83.5	94.5, 94.9	94.6, 93.0
Larynx (C32)	97.9, 98.0	90.3, 66.7	95.7, 75.0	86.4, 87.5	93.6, 100.0
% Death Certificate Only for Males, Females*					
Oral cavity/pharynx (C00-14)	0.4, 0.9	2.5, 3.2	7.0, 6.6	0.0, 0.8	1.6, 0.8
Larynx (C32)	0.6, 0.8	1.1, 0.0	0.0, 0.0	4.5, 0.0	0.0, 0.0

* as reported by IARC CI5 volume X

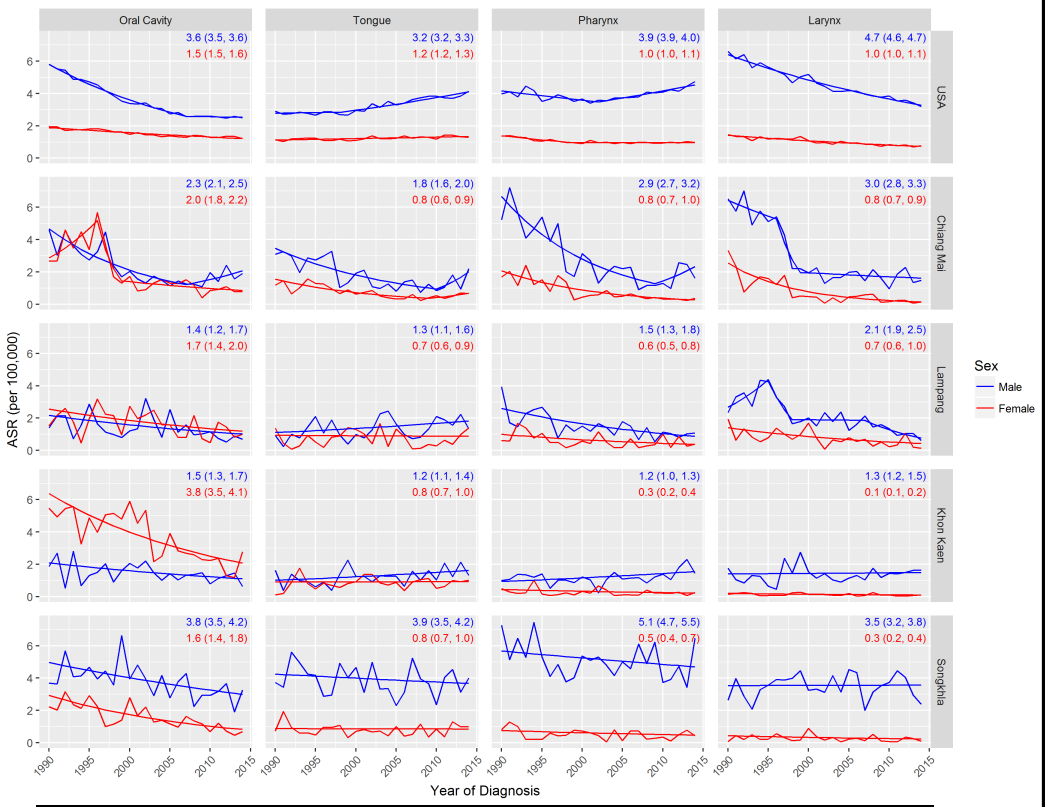
◊ Sources:

A: Pathology labs
B: Public hospital inpatient records
C: Private hospital inpatient
D: Death certificates
E: Radiotherapy depts
F: Oncology depts
G: Public hospital discharge records
H: Private hospital discharge records
I: Imaging facilities
J: Hematology labs
K: Hospice/palliative units
L: Public hospital outpatient records
M: Private hospital outpatient records
N: General practitioner records
O: Health insurance records
P: Autopsy services

Figure 5.1: Map of Thailand: Inclusion of Four Cancer Registries

Figure 5.2. Subsite Joinpoint analyses of HNSCC trends by sex and registry. A, expected age standardized rate ($EASR_{1990-2014}$) and 95% confidence intervals. B, Annual percent change (APC) and 95% confidence intervals.

A



B

Oral Cavity		Tongue		Pharynx		Larynx		
1990-2007	-4.7* (-5.0, -4.3)	1990-1998	0.2 (-1.5, 2.0)	1990-2002	-1.5* (-2.3, -0.6)	1990-2014	-2.8* (-3.0, -2.5)	USA
2007-2014	-0.3 (-1.6, 1.1)	1998-2014	2.4* (1.9, 2.9)	2002-2014	2.2* (1.5, 3.0)	1990-2014	-2.7* (-3.2, -2.3)	
1990-2014	-1.8* (-2.1, -1.5)	1990-2014	0.8* (0.4, 1.2)	1990-1999	-4.1* (-5.6, -2.6)			
				1999-2014	0.1 (-0.6, 0.8)			
1990-2007	-7.6* (-9.9, -5.2)	1990-2010	-6.3* (-8.5, -4.0)	1990-2009	-8.3* (-10.6, -6.0)	1990-1996	-3.2 (-10.1, 4.1)	Chiang Mai
2007-2014	8.0 (-2.2, 19.2)	2010-2014	20.9 (-5.5, 54.8)	2009-2014	12.7 (-9.8, 40.9)	1996-1999	-28.0 (-59.6, 28.3)	
1990-1996	10.3 (-1.1, 23.1)	1990-2009	-7.3* (-9.7, -4.8)	2000-2014	-8.2* (-9.9, -6.4)	1990-3014	-1.3 (-3.9, 1.4)	
1996-1999	-34.6 (-68.3, 34.7)	2009-2014	13.3 (-5.5, 35.9)			1990-2014	-11.2* (-13.7, -8.7)	
1999-2014	-3.4 (-6.9, 0.3)							
1990-2014	-3.1* (-5.8, -0.3)	1990-2014	2.1 (-0.4, 4.7)	1990-2014	4.5* (-6.1, -2.8)	1990-1995	10.4 (-2.4, 24.9)	Lampang
1990-2014	-3.1* (-5.4, -0.8)	1990-2014	-0.3 (-4.0, 3.5)	1990-2014	-4.0* (-7.0, -0.9)	1995-1998	-24.3 (-54.9, 27.0)	
						1998-2007	-0.2 (-7.0, 7.0)	
						2007-2014	-11.8* (-19.3, -3.5)	
						1990-2014	-4.8* (-7.7, -1.9)	
1990-2014	-2.6* (-4.5, -0.7)	1990-2014	2.0 (-0.1, 4.2)	1990-2014	2.1* (0.4, 3.8)	1990-2014	0.2 (-1.6, 2.1)	Khon Kaen
1990-2014	-4.6* (-6.0, -3.1)	1990-2014	0.1 (-2.2, 2.5)	1990-2014	-2.9 (-6.5, 0.8)	1990-2014	-3.0 (-6.3, 0.4)	
1990-2014	-2.1* (-3.4, -0.8)	1990-2014	-0.6 (-1.9, 0.7)	1990-2014	-0.8 (-1.9, 0.4)	1990-2014	0.0 (-1.3, 1.3)	Songkhla
1990-2014	-5.1* (-6.8, -3.4)	1990-2014	-0.1 (-2.4, 2.2)	1990-2014	-2.0 (-4.8, 0.9)	1990-2014	-2.5 (-6.4, 1.5)	

Chapter 6

Increasing Prevalence of HPV in Oropharyngeal Carcinoma Suggests Adaptation of p16 Screening and De-escalation of Treatment in Southeast Asia

Abstract

Background: Human papillomavirus (HPV) has been etiologically linked to increasing oropharyngeal squamous cell carcinoma (OPSCC) rates in much of the Western world. Nevertheless, the role of HPV in Southeast Asia, a high incidence region for head and neck cancer, has not been assessed.

Methods: 107 formalin-fixed, paraffin-embedded (FFPE) tissue blocks and corresponding patient data were obtained from Srinagarind Hospital in Khon Kaen, Thailand. The samples were genotyped using polymerase chain reaction (PCR) and stained for p16. Patient characteristics between HPV+ and – patients were compared using chi-squared and t-test. Inverse probability weights were created based on data from the hospital-based cancer registry and used in all further statistical analyses. Diagnosis year, age, annual sample size and sex adjusted linear regression was used to assess changes in OPSCC HPV prevalence across time and to create projections. Kaplan-Meier estimation and cox proportional hazard models were used to determine survival differences between OPSCC patients based on HPV status.

Results: 14 patients exhibited monoinfection with HPV16, two with HPV18 and one was co-infected with both HPV16 and 18. PCR results were in complete agreement with p16 staining. On average, HPV+ patients were younger (p-value: 0.05) and more likely to have tonsil cancer (p-value: 0.002). HPV prevalence increased at a rate of 2% annually (p-value: 0.01), from 16% in 2012 to 26% in 2017. At the current rate, OPSCC HPV positivity is expected to exceed 50%

by 2030. HPV positivity was shown to be protective in both Kaplan-Meier (log-rank p=0.02) and sex, age and stage adjusted cox proportional hazard models (HR: 0.34 [95%CI: 0.22, 0.52]).

Conclusion: Given the increased prevalence and similarities in presentation of HPV+ OPSCC to those observed in Western countries, we believe it is reasonable to suggest the adaptation of p16 staining and subsequent restaging of OPSCC tumors as suggested by the American Joint Committee on Cancer in Southeast Asia.

Introduction

Head and neck cancer (HNC) is comprised of a heterogeneous group of neoplasms that span several anatomical subsites including the: oral cavity, oropharynx, hypopharynx, nasopharynx, larynx, salivary glands, paranasal sinuses and nasal cavity. Taken together, HNC is considered to be the sixth most common cancer worldwide[1] with the highest incidence reported in South and Southeast Asia[2,3]. Although variable by subsite, ~90% of HNCs are morphologically characterized as squamous cell carcinoma (HNSCC)[4] for which the primary risk factors are well established and include heavy alcohol consumption, use of tobacco products (both combustible and chewing) and betel quid. Despite reductions in the use of tobacco products, numerous studies have noted rising incidence in oropharyngeal squamous cell carcinoma (OPSCC); these increases have been identified to be etiologically linked to persistent infection with high risk human papilloma virus (HPV), resulting in a clinically and epidemiologically distinct subset of OPSCC[5,6].

HPV-positive and -negative OPSCCs are distinctly different in both their presentation and response to treatment. Oropharyngeal squamous cell carcinomas are associated with the lymphoid mucosae of Waldeyer's ring, and consequently arise from the soft palate, base of the tongue, tonsils, and oropharynx[7,8]. These sites are characteristically lined with non-keratinizing stratified squamous epithelium that is folded to create basal keratinocyte filled crypts that trap antigens and allow for nuclear integration of HPV DNA[7,9]. The resulting malignancies are often poorly differentiated and possess a non-keratinized basaloid morphology[10-12]. In the Western world, HPV-positive OPSCC patients tend to be younger, higher socio-economic status and are less likely to partake in high risk behaviors typically associated with HNC[13-16]. Due to transmission modalities of HPV, sexual behaviors, particularly regarding age at sexual debut and number of sexual partners, are especially relevant in understanding risk and incidence of this cancer subtype[17,18]. While historically best described in cervical cancer, 14 of the more than 100 HPV genotypes are considered to be "high risk" due to their oncogenic potential[19]. Of these, HPV16 and 18 are most frequently implicated in malignant transformation, with HPV16 accounting for more than 90% of HNSCC cases[9,20]. In persistent infection, the HPV oncoprotein E7 competes with the E2F transcription factor for binding to the retinoblastoma protein, inactivating it and causing uncontrolled cellular proliferation; this disruption in the cellular cycle

subsequently results in the overexpression of tumor suppressor protein p16[21]. Due to this phenomenon, immunohistochemical detection of p16 is commonly used as a surrogate marker for biologically active HPV infection. Numerous studies have shown HPV status to be a strong independent prognostic factor for survival among OPSCC patients[22,23], leading the American Joint Committee on Cancer to suggest restaging these tumors to allow for less aggressive treatment[24].

Despite its important prognostic significance, the vast majority of studies investigating the role of HPV in HNC have been conducted in Western countries, limiting generalizability and adaptation of screening and treatment methodologies on a global scale. Thailand has undergone rapid socioeconomic development in the last decade, allowing for improved control of communicable diseases and resulting in the emergence of cancer as the leading cause of mortality [25]. Located centrally in Southeast Asia, the sociocultural factors in Thailand are similar to those in the surrounding countries, and while a few studies have implicated HPV as a relevant etiological factor in this setting [26,27], to date only anecdotal evidence has been provided. In this study, we present a temporal molecular and characteristic assessment of HPV prevalence in Thai OPSCC in hopes of informing clinical practice and improving treatment and quality of life among afflicted patients in Southeast Asia.

Methods

Patient and Tissue Selection

Oropharyngeal carcinoma patients diagnosed between 2012 and 2017 were retrospectively identified through the Khon Kaen Cancer Registry[28]. Those individuals under the age of 18 or who had not received biopsies or surgical resection at Srinagarind Hospital in Khon Kaen, Thailand were excluded and the resulting list was sent to the pathology department. One formalin-fixed, paraffin-embedded (FFPE) tissue sample was obtained for each eligible patient for whom tissue was stored in the pathology department's biobank. Pathology records corresponding to each FFPE block were provided for more detailed assessment of the cancer site, stage and source of the sample (resection or biopsy). Additional variables including diagnosis

date, sex, age, stage, survival status and date last seen were obtained from the registry system. Once collected, both the FFPE blocks and corresponding data for the 107 patients were sent to the University of Michigan for further molecular and statistical assessment. OPSCC patient data for the corresponding years, regardless of age or tissue availability, was provided from the Srinagarind Hospital Cancer Registry to check for generalizability and included the following variables: diagnosis date, topography, stage, sex, age, postcode, survival status and date last seen.

Laboratory Analyses

Seven 4-micron sections were cut from each tumor block: 1 slide for H&E staining, 2 slides for p16 staining, and 4 slides for DNA isolation. H&E slides were reviewed by an expert pathologist in head and neck pathology (JM) for % cellularity, % necrosis, and tumor area.

Four unstained sections from each block were placed on uncharged slides for microdissection. Areas of tumor circled on the H&E slide were aligned with the section on the unstained slides. These areas were microdissected for DNA extraction. Input for extraction varied based on the size of the tumor area (mm^2) based on kit recommendations (up to 100 mm^2 total). DNA was extracted per manufacturer guidelines using the DNAStorm FFPE Kit (Cell Data Sciences Inc., USA) including RNase treatment. Isolated DNA was stored at -20 C until further analysis.

All samples were evaluated for HPV status among 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using the 14 High-risk HPV with 16/18 Genotyping Real-Time Polymerase Chain Reaction (PCR) kit (HBRT-H14 kit, Hybribio Limited). Four distinct fluorophores were utilized in this kit which allowed for identification of HPV-16 (HEX), HPV-18 (ROX), 12 other high-risk types of HPV (FAM), and an internal control (Cy5) present within each sample. Real-time PCR was performed following manufacturer instructions. Briefly, PCR master mix, DNA Taq polymerase were combined to create a PCR mix. To bring the total reaction volume to 20 μL, 2 uL of template DNA was added to each well. One positive control and one negative control was included on each plate. Samples were evaluated using thermocycler conditions provided by the manufacturer on the BioRad CFX96.

Results were assessed using the Bio-Rad CFX Manager Version 1.5. The baseline threshold was set from 3-15 cycles, as directed by the kit instructions. Positive controls were valid when the threshold cycle (Ct) ≤36, while negative controls were valid when undetected. Samples were re-run if either control was deemed invalid on the plate. Due to noise in the signal across samples, we did not evaluate the FAM fluorophore. This restricted our results to only HPV-16 and HPV-18. Positive HPV-status was determined when Ct ≤35, while negative samples did not amplify and had undetected Ct values.

Immunohistochemical staining was performed on the DAKO Autostainer (Agilent, Carpinteria, CA) using Envision+ and diaminobenzadine (DAB) as the chromogen. De-paraffinized sections were labeled with p16 ^{Ink4a} (Abcam, Cambridge, MA, Ab-108349 Clone EPR1473, 1:2000) for 30 minutes at ambient temperature. Heat induced epitope retrieval using 10 mM citrate buffer pH6.0 was used prior to staining. Appropriate negative (no primary antibody) and positive controls (cervix) were stained in parallel with each set of slides studied. Slides were scored for p16 status by JM to distinguish between p16 negative (no staining or missing staining from either the nuclei or cytoplasm) and p16 positive (staining of both nuclei and cytoplasm within ≥70% of the tumor tissue) samples.

Worst pattern of invasion (WPOI) was assessed by a pathologist (JM) using H&E slides on scale of 1-5 according to previously published guidelines[29-31]. In brief, the five categories were defined as follows: type 1=pushing border, type 2=finger-like growth, type 3=large separate islands of >15 cells per island, type 4=small discontiguous tumor islands with ≤15 cells, and type 5= discontiguous tumor satellites >1mm from the main tumor or next closest satellite. In-situ tumors or small biopsy sections lacking margins that could not be properly scored were considered to be indeterminate.

Statistical Analysis

Descriptive statistics comparing HPV positive and negative patients were conducted using chi-squared test (for categorical variables) and t-test (for continuous variables). Upon review of the pathology records, 11 samples were determined to be oral cavity and excluded from further

analyses. A similar analysis was completed to assess differences between our sub-cohort of patients and the larger Srinagarind Hospital cancer registry. To account for differences observed between these groups, we utilized inverse probability weighting in order to allow our subcohort patients to better represent the larger registry cohort, thereby improving generalizability[32]. Propensity scores were produced using baseline demographic variables, which included: age at diagnosis, sex, disease site and year of diagnosis. The reciprocal of the propensity score was used as the weight in further statistical analyses. To evaluate trends in HPV prevalence, proportions, calculated as the number of HPV+ cases over the sum of all OPSCC cases diagnosed within a given year, were modeled using linear regression as a function of year of diagnosis, age, annual sample size and sex. To create a parsimonious model, covariates were selected based on a-priori knowledge and backward selection. Projections were modeled based on the multivariate linear regression model. A Kaplan-Meier plot was generated to assess crude survival differences between HPV positive and negative patients and compared using a log-rank test. Finally, unadjusted as well as sex, age and stage adjusted cox proportional hazard (HR) models were employed to further evaluate survival variability based on HPV status. All statistical tests were conducted using SAS software 9.4 (SAS Institute, Inc.).

Results

Of the 107 patient samples tested, 17 were found to have to be positive for HPV, while 90 tested negative. HPV positivity was found only in the oropharynx, with zero cases of oral cavity cancer exhibiting viral DNA or p16 positivity. Among those with HPV+ OPSCC, fourteen were monoinfected with HPV16, two monoinfected with HPV18 and one was co-infected with both HPV16 and 18. PCR HPV testing results were in complete agreement with p16 staining. When comparing HPV positive and negative patients, HPV+ patients were, on average, slightly younger, with a mean age of 54.94 (p-value: 0.05), and were more likely to have tonsil cancer, which comprised 76.47% of all HPV+ tumors (p-value: 0.002). Males made up the vast majority of the sample size regardless of HPV status, and we found no significant differences in stage, year of diagnosis, worst pattern of invasion or mean survival time between groups (**Table 6.1**). When comparing our sub-cohort to the larger hospital-based cancer registry of OPSCC patients,

significant differences were found for cancer site (p-value: <0.0001), stage (p-value: <0.0001) and year of diagnosis (p-value: 0.0003) (**Table 6.2**).

Linear regression results assessing changes in HPV prevalence over time, adjusting for age, sex, annual sample size and year of diagnosis can be found in **Figure 6.2**. HPV prevalence significantly increased at a rate of 2% annually (p-value: 0.01), starting at 16% in 2012 and increasing to 26% in 2017. Assuming prevalence continues at this rate, OPSCC HPV positivity is expected to exceed to 50% by 2030.

Survival analysis results can be found in **Figure 6.3**. HPV positivity was shown to elicit a strong survival benefit in Kaplan-Meier estimation (log-rank p=0.02). Cox proportional hazard model results demonstrated a marginally significant protective effect of HPV positivity in crude models (HR: 0.79 [95%CI: 0.62, 1.02]), but a statistically significant 66% reduced risk of overall mortality after adjusting for sex, age and stage (HR: 0.34 [95%CI: 0.22, 0.52]). No significant association was found between WPOI and HPV status or survival (data not shown).

Discussion

This is the first study to present temporal molecular and characteristic assessment of HPV in oropharyngeal squamous cell carcinoma samples from Southeast Asia. The results of these analyses provide strong evidence of an increasing prevalence of high-risk HPV in OPSCC, as well as a clear survival benefit conferred by the presence of this virus. In light of these findings, together with the agreement between p16 staining and PCR results and the cost effectiveness of this screening model, we believe it is reasonable to suggest that the American Joint Committee on Cancer guidelines for screening and downstaging of treatment be adapted in this setting.

As socioeconomic growth increased throughout Thailand, pushing the country through a rapid epidemiologic transition, the country saw cancer related mortality double from an age-standardized rate (ASR) of 48.4 per 100,000 in 1998 to 95.2 per 100,000 in 2011[33]. Head and neck cancer is ranked among the top five more commonly diagnosed malignancies in Thailand, with age standardized incidence rates of 15.7 and 10.7 per 100,000 among males and females,

respectively [33]. Oropharyngeal carcinoma incidence in particular was recently shown to be increasing across Thailand, suggesting that the etiological shift to HPV+ OPSCC observed in much of the Western world may also be similarly occurring in Southeast Asia[26]. Nevertheless, despite the county's high HNC burden, little is known regarding the role of HPV as a potential etiological factor in OPSCC. Several studies have attempted to elucidate head and neck HPV prevalence in the Thai populating with disparate results. Khovidhunkit at al. examined 65 oral squamous cell carcinoma, leukoplakia and lichen planus biopsies from Rajvithi Hospital and Mahidol University in Bangkok for the presence of HPV using PCR and found only one sample to exhibit HPV DNA[34]. Another study conducted in Northeastern Thailand found HPV positivity in 29.7% of exfoliated cells from oral squamous cell carcinoma (OSCC) cases, compared to 13% in controls[35]. Finally, a third study examining 48 oral cavity and 4 oropharyngeal carcinomas from Srinagarind hospital found 40.8% of OSCC and 50% of OPSCC cases to possess HPV E6/E7 mRNA using in situ hybridization (ISH). While we did not find any evidence of HPV in our oral cavity samples, 17 of the 96 OPSCC were shown to exhibit HPV DNA as well as p16 positivity.

In accordance with decreasing smoking rates[36,37] and changes in cultural norms around sexual behaviors[18,38], numerous studies have proposed that the steadily rising incidence rates of OPSCC may be tied to increased HPV prevalence[39]. A study from the Netherlands showed increases in HPV+ OPSCC from 5% in 1990 to 29% in 2010[40], while Australia saw a rise from 19% in 1987-1990 to 47% for 2001-2005[41]. In the United States, Chaturvedi et al. demonstrated a significant increase from 16.3% in 1984-1989 to 72.7% by 2000-2004[6]. While this etiological shift has formerly been proposed to effect predominantly Western, more developed countries[42], the results of this study provide evidence that Southeast Asia is beginning to similarly experience an upsurge in HPV related OPSCC. Among those patients seen at Srinagarind Hospital, whose catchment area can be found in **Figure 6.1**, HPV prevalence increased at a rate of 2% from 16% in 2012 to 26% in 2017. If this trend continues at the current rate, Thailand is expected to have an OPSCC HPV prevalence over 50% by 2030 (**Figure 6.2**).

The presentation and behavior of these tumors appears to be similar to those reported in Western countries, with younger males bearing the greatest cancer burden[42]. In regard to HPV strain, 88%

of HPV+ OPSCC cases testing positive for HPV16, while the remainder possessed HPV18 DNA. It is generally accepted that HPV positivity predicts improved survival among OPSCC patients[23]. In accordance with the literature, we found HPV status to confer a strong protective effect in our cohort, even after adjustment for sex, age and stage.

While PCR and ISH methods are typically used to diagnose HPV, they are not widely available in diagnostic laboratories and do not distinguish between integrated and non-integrated genomes[21]; consequently, p16 staining is commonly used as a surrogate marker for the presence of viral oncoproteins associated with HPV infection. The p16 immunohistochemical stain is nearly universally available and technically less laborious, allowing costs to be an estimated 2-16 times lower than HPV-specific tests[43]. In this study we verified the utility of p16 staining as a viable proxy measure for high risk HPV in Thailand, with 100% agreement between the PCR and p16 staining results.

Due largely to the retrospective nature of the study, the results should be interpreted in light of several limitations. FFPE blocks were obtained based upon availability within the biobank, limiting the sample size, particularly in earlier years; nevertheless, registry data was used to create inverse probability weights to allow for improved representation and generalizability for statistical analyses. Additionally, depletion of FFPE blocks through repeated use may have limited the ability to detect tumor tissue characteristics. If applicable, this would likely result in an underestimation of HPV prevalence as available tumor tissue is often reduced through subsequent cuts. While the role of HPV within the oral cavity is still largely debated[44], and not a primary aim of this manuscript, the limited sample size of oral cavity tumors available in this study preclude us from making any conclusions regarding HPV prevalence within this HNC subtype. Because the majority of the available patient data was obtained through hospital registry records and not abstracted from patient files, adjustment for potential confounders such as smoking, alcohol and betel quid use were not possible. Additional survival analyses based on treatment modality were also unachievable and comorbidities could not be accounted for in the model.

In light of the increasing prevalence of high risk HPV in OPSCC, in addition to the similarities in presentation of these cases to those observed in Western countries, we believe it is reasonable to suggest the adaptation of p16 staining, corresponding restaging and treatment modalities as suggested by the American Joint Committee on Cancer[24] in Southeast Asia. While further studies need to be conducted in order to validate these results, implementing p16 staining as the standard of care for this increasingly common cancer type[26] would allow for improved monitoring and more informed treatment decisions. In conclusion, screening and subsequent de-escalation of treatment would likely allow for decreased mortality and improved quality of life among afflicted patients in Southeast Asia.

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Table 6.1: Patient Characteristics of HPV Positive and Negative Cases (2012-2017)

	HPV Positive (N=17)	HPV Negative (N=90)	p-value
Mean age (SD), [range]	54.94 (7.67), [42-73]	56.63 (11.97), [36-81]	0.05
Male, N (%)	13 (76.47)	71 (78.89)	0.82
Cancer Site, N (%)			0.002
Base of tongue	3 (17.65)	38 (42.22)	
Tongue*	0 (0.00)	9 (10.00)	
Hard palate*	0 (0.00)	1 (1.11)	
Soft palate	1 (5.88)	17 (18.89)	
Mandible*	0 (0.00)	1 (1.11)	
Pharynx	0 (0.00)	5 (5.56)	
Tonsil	13 (76.47)	19 (21.11)	
Stage, N (%)			0.37
1	0 (0.00)	6 (6.67)	
2	1 (5.88)	10 (11.11)	
3	3 (17.65)	22 (24.44)	
4	13 (76.47)	47 (52.22)	
Missing	0 (0.00)	5 (5.56)	
Year of Diagnosis, N (%)			0.15
2012	2 (11.76)	3 (3.33)	
2013	1 (5.88)	11 (12.22)	
2014	2 (11.76)	29 (32.22)	
2015	4 (23.53)	26 (28.89)	
2016	4 (23.53)	12 (13.33)	
2017	4 (23.53)	9 (10.00)	
Source of Sample			0.13
Biopsy	11 (64.71)	73 (81.11)	
Excision	6 (35.29)	17 (18.89)	
Worst Pattern of Invasion, N (%)			0.42
1	4 (23.53)	8 (8.89)	
2	6 (35.29)	35 (38.89)	
3	3 (17.65)	22 (24.44)	
4	3 (17.65)	18 (20.00)	
5	0 (0.00)	4 (4.44)	
Indeterminate	1 (5.88)	3 (3.33)	
Median Survival Time in Days	521.00	376.50	0.59
*** Denotes oral cavity disease sites**			

Table 6.2: Oropharyngeal Patient Characteristics

	Sub-Cohort (N=96)	Registry Cohort (N=243)	p-value
Mean age (SD), [range]	56.41 (11.44), [36-81]	55.98 (12.81), [25-91]	0.20
Male, N (%)	79 (82.29)	179 (73.66)	0.09
Cancer Site, N (%)			<.0001
Base of Tongue	41 (42.71)	74 (30.45)	
Oropharynx*	23 (23.96)	25 (10.29)	
Tonsil	32 (33.33)	144 (59.26)	
Stage, N (%)			<0.0001
1	5 (5.21)	9 (3.70)	
2	9 (9.38)	25 (10.29)	
3	23 (23.96)	42 (17.28)	
4	54 (56.25)	79 (32.51)	
Missing	5 (5.21)	88 (36.21)	
Year of Diagnosis			0.0003
2012	4 (4.17)	38 (15.64)	
2013	12 (12.50)	62 (25.51)	
2014	24 (25.00)	53 (21.81)	
2015	27 (28.13)	31 (12.76)	
2016	16 (16.67)	32 (13.17)	
2017	13 (13.54)	27 (11.11)	
*** Comprised of cancers of the pharynx and soft palate**			

Figure 6.1: Distribution of Oropharyngeal Carcinoma Cases Seen at Srinagarind Hospital

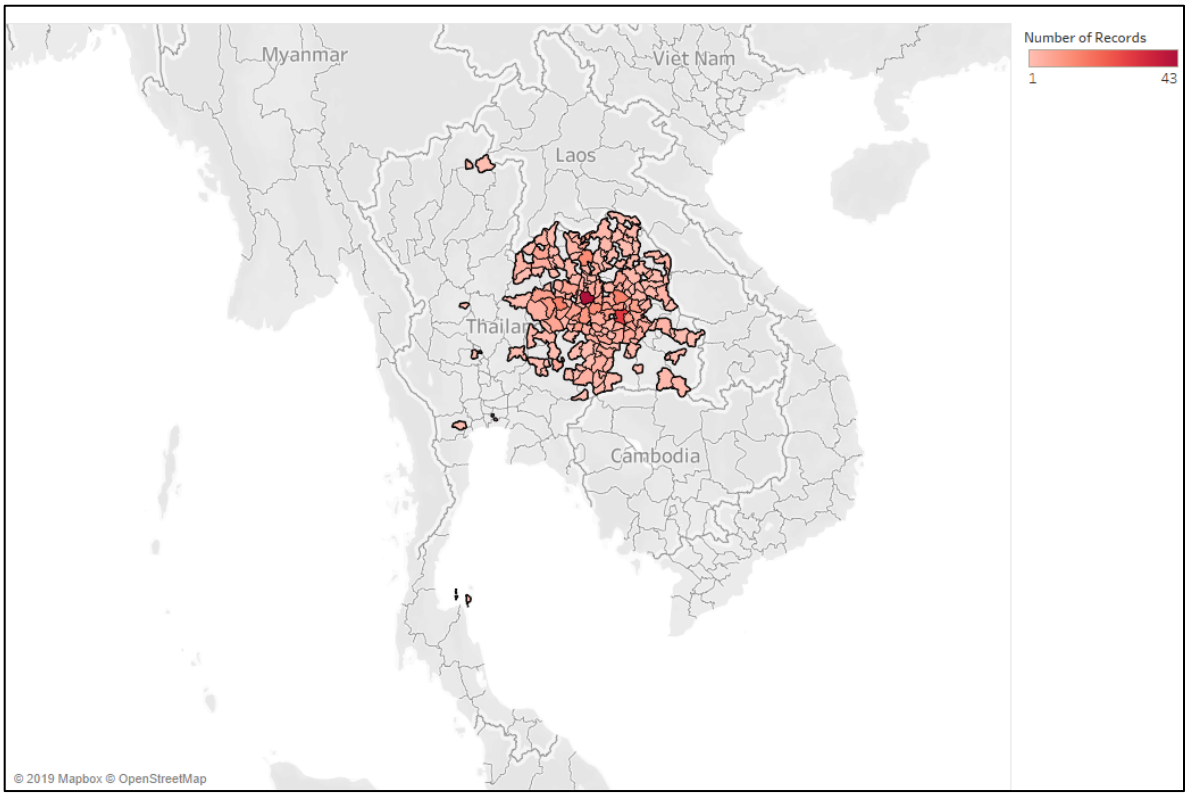


Figure 6.2: Temporal HPV+ Oropharyngeal Prevalence

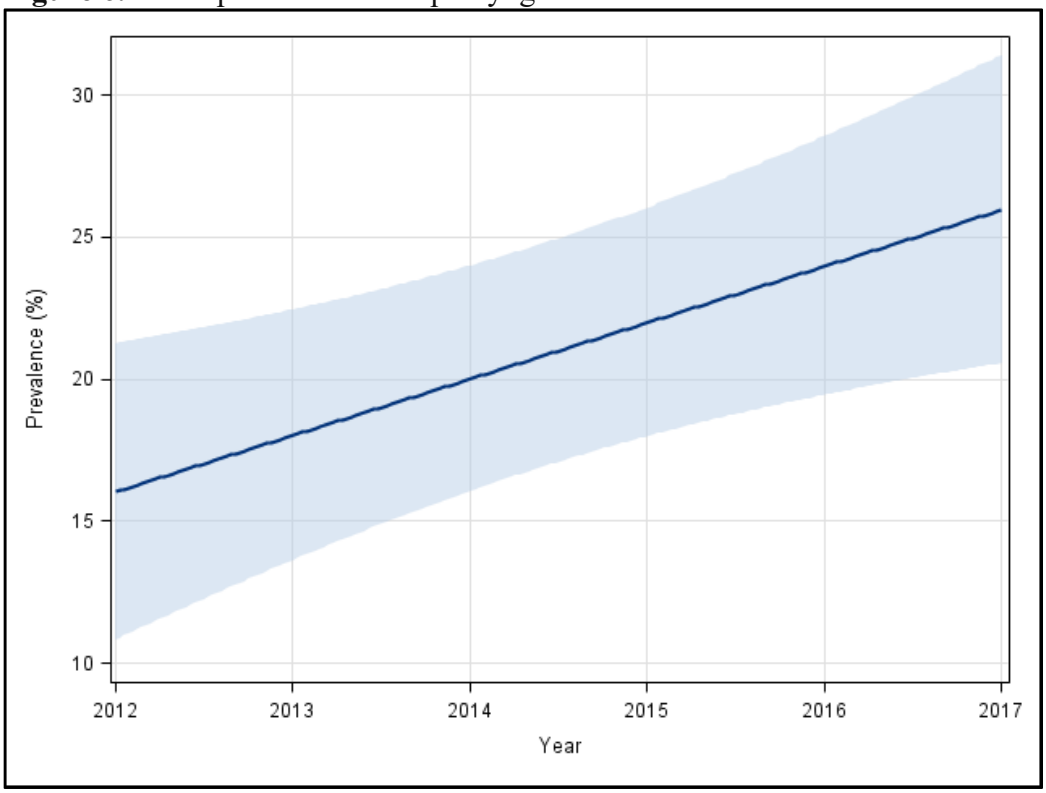
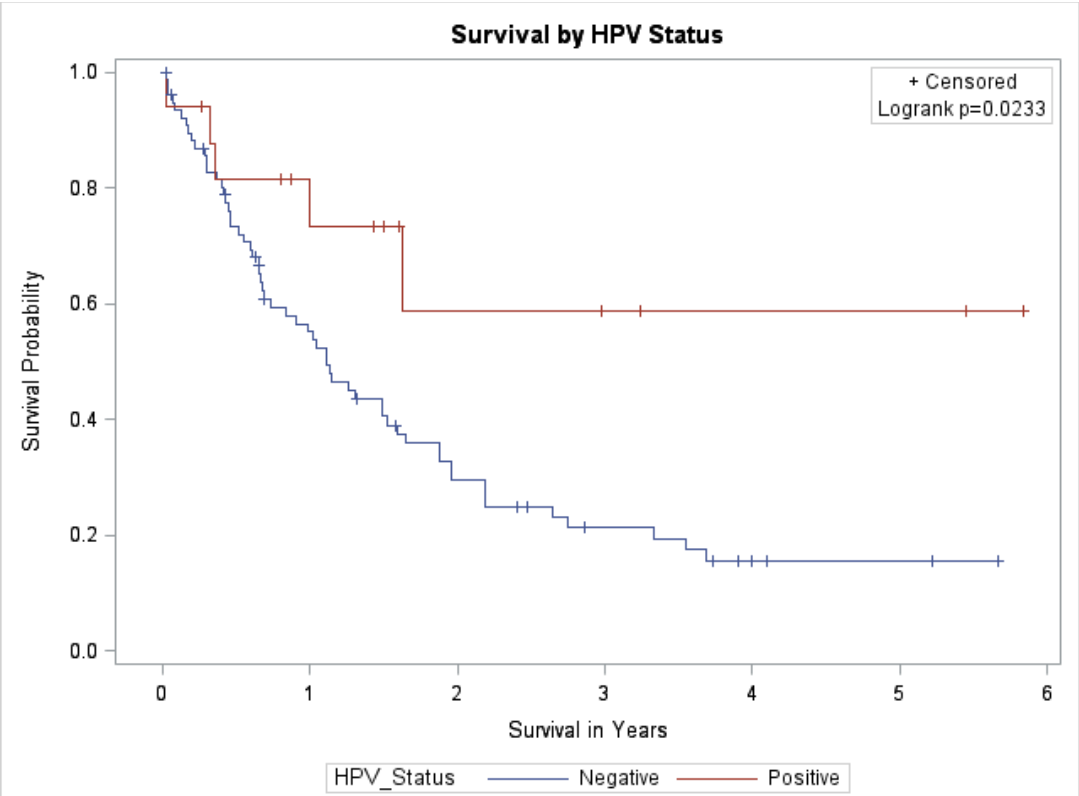


Figure 6.3: Survival Analyses for HPV-positive and -negative OPSCC Patients



	HR (95% CI) Unadjusted	HR (95%CI) Adjusted*
HPV Negative	(Ref)	(Ref)
HPV Positive	0.79 (0.62,1.02)	0.34 (0.22, 0.52)
*** Adjusted for sex, age and stage**		

Chapter 7

Pretreatment Diet, Serum Carotenoids and Tocopherols Influence Tumor Immune Response in Head and Neck Squamous Cell Carcinoma

Abstract

Background: Tumor infiltrating lymphocytes (TILs) have recently emerged as an important factor in informing treatment decisions for head and neck squamous cell carcinoma (HNSCC). Nevertheless, little is known about the role of modifiable risk factors, such as diet, on TILs.

Methods: Immunohistologic expression of CD4, CD8, CD68, CD103, CD104 and FOXP3 were assessed in tissue microarrays from 233 previously untreated HNSCC patients. Associations between these markers and pre-treatment dietary patterns were then evaluated using linear regression models. Logistic regression models assessing the associations between baseline serum carotenoids, tocopherols and TILs were conducted on a smaller subset of 70 patients. Cox proportional hazard models were used to evaluate the joint association between diet and TILs on overall and recurrence free survival.

Results: High intake of a Western dietary pattern decreased CD8+ and FOXP3+ infiltrates after adjustment for age, sex and batch (p-value: 0.03 and 0.02, respectively). Multivariate logistic regression models demonstrated significant increases in CD8+ (OR: 2.21; p-value: 0.001) and FOXP3+ (OR: 4.26; p-value: <0.0001) among patients with high gamma tocopherol. Conversely, high levels of xanthophylls (OR: 0.12; p-value:<0.0001), lycopene (OR: 0.36; p-value: 0.0001) and total carotenoids (OR: 0.31; p-value: <0.0001) significantly decreased CD68+. Among those with high CD4+ (HR: 1.77; p-value: 0.03), CD68+ (HR: 2.42; p-value: 0.004), CD103+ (HR: 3.64; p-value: 0.03) and FOXP3+ (HR: 3.09; p-value: 0.05) infiltrates, having a high Western

dietary pattern significantly increased the risk of overall mortality when compared to those with a low Western dietary pattern, even after adjusting for age, sex, stage, disease site, HPV status and TMA; a similar effect was found between the high Western dietary pattern and FOXP3+ (HR: 2.93; p-value: 0.0002) on recurrence free survival in fully adjusted models.

Conclusion: Dietary patterns and serum carotenoids may play an important role in modifying TILs, and ultimately, outcome after diagnosis with HNSCC. The results of this study could inform dietary interventions among high risk individuals in an attempt to moderate risk and improve HNSCC prognosis.

Introduction

Head and neck cancer (HNC) encompasses malignant lesions found within the epithelial tissue of the oral cavity, pharynx, larynx and nasopharynx, together comprising the sixth most common cancer worldwide [1]. More than 90% of neoplasms within the head and neck are reported to be squamous cell carcinoma (HNSCC), commonly associated with extensive exposure to alcohol and tobacco use [2]; however, decreases in tobacco consumption in the United States (US)[3,4] has led to the implication of high-risk human papillomavirus (HPV) as the primary etiologic factor associated with oropharyngeal carcinoma [5]. Irrespective of advances in treatment, HNC has experienced a relatively stagnant five-year relative survival rate of 40-50% [6], compelling the need for further research and understanding of this heterogenous disease.

Recent studies on the role of tumor-infiltrating lymphocytes (TILs) and cancer suggest tumorigenesis to be an immunologic disease as well as genetic one. HNC tumors are unique in that they lie within close proximity to lymphoid tissue in the Waldeyer's ring, therefore allowing for dense infiltration of immune cells to be easily integrated into the lesion [7]. Several studies assessing the role of TILs in HNC have suggested these immune markers to be strong prognostic tools to guide treatment decisions [7]. HPV+ HNSCC patients commonly display nodal metastasis, yet the overall and progression-free survival rates are typically better than those among their HPV- counterparts. Due largely to reports of dense lymphocytic infiltrates in HPV+ oropharyngeal carcinoma, one proposed rationale for the observed survival benefit is that the body's immune response against the viral antigen, particularly in the form of CD8+ and FOXP3+, may aid in driving the elimination of the tumor cells [8-13]. In accordance with this research, the American Joint Committee on Cancer has recently suggested restaging HPV+ HNSCC in an attempt to curb treatment-related morbidity and mortality rates among afflicted patients [14]. TILs have also been repeatedly noted as an important indicator in maximizing treatment efficacy, with increased CD3+, CD4+, CD8+ and FOXP3+ T-cell infiltrates correlated with improved survival among HNSCC chemoradiation therapy patients[15-17].

Numerous epidemiologic studies have demonstrated a bi-directional effect of diet on HNSCC risk, progression and prognosis[18-27]. While poor nutrition is believed to account for an estimated

third of all cancer related deaths in the US[28,29], the molecular mechanisms underlying the protective effects of bioactive dietary agents remains to be fully elucidated. Immune system modulation through dietary components that can mediate inflammation within the tumor microenvironment is an important mechanism that has been understudied in HNSCC. Although pro-inflammatory factors are essential in the biological response to trauma or infection, excessive production of cytokines, chemokines, growth factors, prostaglandins, and reactive oxygen and nitrogen species have been shown to impact tumor initiation and progression by increasing mutation and cellular proliferation rates, decreasing apoptosis and facilitating angiogenesis [30,31]. Increased consumption of fruits and vegetables have consistently been shown to improve HNSCC prognosis, possibly by counteracting many of the aforementioned inflammatory products through their antioxidant, antimutagenic and antiproliferative properties [32,33]. Nevertheless, no studies to date have assessed the role of diet on anti-tumor immune response in HNC.

With increasing interest in the role of tumor immune response in personalized medicine and immunotherapy, it is important to assess the potential role of diet on tumor immune response and, ultimately, outcome. The results of this study will not only impact our knowledge regarding disease pathogenesis, but also aid in facilitating a conversation about dietary recommendations for both HNSCC prevention and intervention.

Methods

Study Population

Incident cases diagnosed with HNSCC were recruited to participate in this prospective cohort study as part of the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE). Upon approval from the Institutional Review Board at the University of Michigan, study participants provided signed, informed consent to participate. Exclusion criteria included being <18 years of age, pregnant, non-English speaking, diagnosed with another non-upper aerodigestive tract cancer, diagnosed with mental instability, or diagnosed with

another head and neck primary within five years preceding the signed consent. In total, 1,042 subjects were enrolled into the study between November 2008 and October 2014.

At baseline, subjects were asked to complete a self-administered health questionnaire that ascertained data on demographic characteristics, tobacco and alcohol use, physical activity, comorbidities, sleep, depression and quality of life. Thirty mL peripheral blood samples were taken prior to treatment using routine venipuncture techniques and formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected from diagnostic biopsies. In order to track patient status, participants were routinely followed every three to six months in clinic. Vital status was reviewed annually following diagnosis. Mortality and recurrence status were obtained by research assistants through medical record review, annual health surveys, the Social Security Death Index and LexisNexis through April 2016. Time to first recurrence was calculated as the number of days between the initial diagnosis at Michigan Medicine and the date of first recurrence. Patients who completed treatment but exhibited persistent disease (were never determined by a medical doctor to be disease-free) were given a recurrence time of 1 day.

For the purpose of this analysis, patients who had not completed their baseline food frequency questionnaire (FFQ) or were not included on a tumor microarray (TMA) were excluded. Additional exclusion criteria included patients who had withdrawn consent, had uncommon tumor sites or unknown primaries, resulting in a final sample size of 233. Finally, for the purposes of the serum carotenoid/tocopherol analysis, those who did not have available baseline serum carotenoid data were excluded, resulting in a sample size of 70.

Specimen and Data Collection

Dietary intake

Dietary intake was collected using the self-administered, semi-quantitative Harvard food frequency questionnaire (FFQ) and analyzed as pretreatment dietary patterns as described previously[25]. In brief, the FFQ was designed to evaluate respondents' typical dietary intake from food and supplements prior to diagnosis. The questionnaire consists of 131 items, and the

reproducibility and validity have both been previously reported [34,35]. The FFQ included standard portion sizes for each item (eg: 1 banana, 3-5 oz chicken), which allowed participants to select their average frequency of consumption over the past year from a list of up to 9 choices ranging from "almost never" to "≥6 times per day." Total energy and nutrient intake were ascertained by summing intakes from each food item based on the selected portion size, reported frequency of consumption, and nutrient content of each food item[34]. Estimations for daily food group servings were calculated by summing the frequency weights of each food item based on reported daily frequency of consumption. Upon exclusion based on missing values and energy intake outliers, 39 foods and food groups were isolated using previously defined methods[25,36,37]. Dietary patterns were deduced by principal component analysis and pattern factor scores were calculated for each patient by summing the reported consumption of the factor food variables weighted by factor loading.

Serum micronutrients

The extraction of serum carotenoids and tocopherols was completed using high-performance liquid chromatography as previously described [38,39]. In summary, equal volumes of serum and ethanol containing butylated hydroxytoluene were mixed and extracted with hexane, utilizing Tocol as the internal standard. To separate carotenoids from tocopherols, a YMC C30 reverse-phase column was used (YMC Company, LTD, Kyoto, Japan) to conduct a gradual elution at 0.2 mL/min total flow on the Shimadzu LC-20AT HPLC system (Shimadzu Corperation, Kyoto, Japan). While detection for carotenoids was at 450 and 472 nm, electrochemical detection using the Coularray electrochemical detector set (Thermo Scientific, Waltham, Mass) was used for Tocol and tocopherols at 310, 390 and 470 mV.

Tumor infiltrating lymphocytes

Hematoxylin-eosin stained slides were created from the aforementioned FFPE tissue specimens and reviewed by an expert pathologist (JM). Upon verification of histology, those slides that expressed >70% cellularity and minimal necrosis were set aside for tissue sampling. Triplicate

0.7mm diameter cores were selected, punched and extracted for each patient to comprise six tissue microarray (TMA) blocks representative of 621 patients.

Slides cut from TMAs were stained for CD4, CD8, CD68, CD103, CD104 and FOXP3 TILs utilizing techniques previously described [15,40]. Briefly, after overnight incubation in a 65°C oven, the sections were deparaffinized, and rehydrated using xylene, graded alcohols, as well as buffer immersion steps. Heat-induced epitope retrieval was then followed by incubation of the slides in a preheated pressure cooker with either Citrate buffer pH6 or Tris-EDTA buffer pH9 and blocked using horse serum. A DAKO autostainer, with chromogens liquid streptavidin biotin horseradish peroxidase and DBA, was then used for immunohistochemical staining. The deparaffinated sections, along with positive and negative controls, were then stained with monoclonal antibodies at the following titrations: CD4-1:250 (Abcam Ab846); CD8-1:40 (Nova Castra VP-C320); CD68 -1:100 (Dako M0814); CD103-1:500 (Abcam Ab129202); CD104 -1:50 (Beta-4 integrin, eBioscience 439-9b); and FoxP3 -1:200 (Abcam Ab20034). The stained slides were digitally imaged to be used in Aperio ImageScope v.12 software (Leica Biosystems).

A technician naive to clinical status scored the stained TMA slides. Grid software (Measure, C Thing Software 2.01) was employed to overlay each tissue core before counting TILs. CD104+ (beta-4 integrin) staining was used before counting TILs to identify the location and extent of carcinoma in each core. Cores were scored as 25%, 50%, 75%, or 100% tumor. Cores deemed to have <25% tumor parenchyma were not scored and therefore excluded from the analysis. Using 200 X magnification, TILs stained with CD4, CD8, CD68, CD103 and FoxP3 antibodies were manually counted. Only those TILs that infiltrated the tumor parenchyma were counted. The TIL count for each core was normalized by dividing each raw value by the fraction of each 0.7 mm core that was indicated as malignant through CD104 staining. Normalizing TIL counts ensured that variation in counted cells was representative of increased TIL density within the tumor parenchyma and not tumor proportion within cores. Normalized mean counts of triplicate samples for each patient were utilized for statistical analysis.

Statistical methods

Descriptive statistics reflective of demographic, clinical and serum carotenoid/tocopherol variables were compared between binary determinants of dietary pattern score using χ^2 (for categorical variables) and t-test (for continuous variables). Due to the fact that participants with dietary data were generally healthier than those missing dietary data, we utilized inverse probability weighting (IPW) in order to allow our subcohort patients to better represent the larger HN-SPORE cohort, thereby improving generalizability[41]. Propensity scores (PS) were generated using baseline demographic variables, including: age at diagnosis, sex, stage, disease site, comorbidity score, drinking and smoking status. The reciprocal of the PS was then used as the IPW in statistical analyses. To construct parsimonious statistical models, covariates believed to be relevant to the analyses were chosen by a priori knowledge and input by treating physicians and determined using backward selection. To assess the association between binary dietary pattern score (around the median) and each continuous TIL marker, both crude and age, sex and TMA adjusted linear regression models were utilized. Logistic regression models were used to evaluate associations between binary serum carotenoids and tocopherols (both individually, and as total groupings); univariate and multivariate models (adjusted for age, sex, disease site and TMA) were compared using Bonferroni correction to account for multiple testing. Finally, Cox proportional hazard (HR) models, adjusted for age, sex, stage, disease site, HPV status and TMA, was used to assess the joint role of dietary pattern and TILs on overall and recurrence-free survival, consequently assessing the potential modification of the relationship between TILs and survival by dietary pattern. All statistical tests were conducted using SAS software 9.4 (SAS Institute, Inc.).

Results

Five year survival among the 233 participants was 73.4%; during the course of the study, 65 (27.9%) of patients died and 56 (24.0%) recurred. The average age at diagnosis was 60.5 (SD: 11.3) years. Most of the study subjects were male (73.4%), white (95.7%), and had some post-secondary education (62.5%). The oral cavity was the most common tumor location (44.2%) and just over half of all patients were diagnosed with stage IV tumors (55.8%). Demographic and clinical characteristics stratified by binary dietary pattern score are shown in **Table 7.1a.** Individuals who consumed higher quantities of the whole foods diet, characterized by high intake

of fruits, vegetables, legumes, whole grains and low-fat dairy, were more likely to be older, more highly educated and former smokers, compared to their counterparts, who were more likely to be current smokers. Males were more likely to consume diets high in red and processed meats, refined grains, potatoes, French fries, high-fat dairy, condiments, desserts, snacks and sugar-sweetened beverages characterized by the Western dietary pattern.

Differences in serum carotenoid/tocopherol concentrations by levels of dietary pattern can be found in **Table 7.1b**. While beta carotene and total carotenoids were the only biomarkers found to be significantly elevated among high vs. low whole foods diet consumers, high Western dietary pattern patients were significantly more likely to have decreased levels of beta carotene, xanthophylls, total carotenoids, as well as alpha-, gamma- and total tocopherol when compared to low Western dietary pattern participants.

Linear regression model results, assessing the association between individual TIL counts and binary dietary pattern (high/low), can be found in **Table 7.2.** While no significant associations were found between any of the TILs and the whole foods dietary pattern, high intake of the Western dietary pattern was consistently associated with decreased CD4+, CD8+, CD103+ and FOXP3+ infiltrates. Significant decreases were noted for CD8+ and FOXP3+ markers, even after adjustment for age, sex and TMA (β: -19.99 and -12.54, respectively).

Serum carotenoids and tocopherols were evaluated for their association with tumor immune response using logistic regression models and significance was determined based on Bonferroni correction (**Table 7.3**). Crude models demonstrated an increased odds of having high CD4+ infiltrates among those possessing high serum alpha tocopherol concentrations (OR: 2.34 [95%CI: 1.59, 3.46]); similar findings were found between alpha tocopherol and CD8+ markers (OR: 2.09 [95%CI: 1.43, 3.06]), as well as CD8+ and alpha carotene (OR: 2.25 [95%CI: 1.53, 3.30]). CD68+ myeloid derived suppressor cells, a TIL marker previously associated with poor HNSCC prognosis when infiltration is high[15], was found to be inversely associated with high serum xanthophylls (OR: 0.21 [95%CI: 0.14, 0.32]), lycopene (OR: 0.53 [95%CI: 0.36, 0.78]) and total carotenoids (OR: 0.46 [95%CI: 0.31, 0.67]); these finding remained significant after adjustment by age, sex, disease site and TMA. Higher levels of serum gamma tocopherol were

found to increase the likelihood of having high CD8+ and FOXP3+ infiltrates in multivariate models (OR: 2.21 [95%CI: 1.37, 3.56]; OR: 4.26 [95%CI: 2.40, 7.58], respectively).

Cox proportional hazard model results for the joint association between binary TILs and binary dietary pattern on overall and recurrence free survival, adjusted for age, sex, stage, disease site, HPV status and TMA, can be found in **Table 7.4** and **7.5.** Surprisingly, among those with high CD4+ TIL counts, having a high whole foods dietary pattern (in reference to a low whole foods dietary pattern) was associated with increased risk of overall mortality (p for interaction: 0.02). Among those with high CD4+ (p for interaction: 0.03), CD68+ (p for interaction: 0.004), CD103+ (p for interaction: 0.03) and FOXP3+ (p for interaction: 0.05) infiltrates, having a high Western dietary pattern significantly increased the risk of overall mortality when compared to those with a low Western dietary pattern. Similar results were found for recurrence-free survival; among those with high CD4+ (p for interaction: <0.0001) and FOXP3+ (p for interaction 0.0002) TILs, having a high Western dietary pattern appeared to increase the risk of recurrence when compared to those with low Western diet consumption.

Discussion

This study is the first of its kind to report that both pretreatment dietary patterns, as well as serum carotenoids, can not only influence tumor immune response, but may also mediate the association between TILs and survival. Consuming a high Western dietary pattern was consistently associated with decreased number of infiltrates found within the tumor, with significant reductions noted for CD8+ and FOXP3+ in multivariate models. Additionally, we found that high serum concentrations of xanthophylls, lycopene and total carotenoids decreased the number of detrimental CD68+ cells in HNSCC tumors, while gamma tocopherol appeared to significantly increase both CD8+ and FOXP3+ lymphocytes. Finally, we demonstrated that the effect the Western dietary pattern exhibits on the tumor microenvironment may have significant negative implications on overall and recurrence-free survival among HNSCC patients.

While numerous studies have analyzed the association between dietary factors and HNSCC [25,42-45], there have been no studies, to our knowledge, that have assessed the role of dietary patterns or

serum carotenoids on tumor immune response at a molecular level. Overall, dietary studies have largely provided evidence that poor nutritional status, often characterized by low consumption of fruits and vegetables, increases the risk of HNSCC[46], while high consumption of nutrient rich foods have the opposite effect[44,45,47]. Smoking and alcohol consumption, two primary risk factors in HNC development, are believed to play an integral role in counteracting the protective effects conferred by dietary nutrients. Tobacco products, combustible and smokeless, have been shown to produce free radicals and reactive oxygen molecules, which have the ability to react with the lipid bilayer, denature proteins, induce DNA damage, and trigger chronic inflammation [30,48]; high alcohol consumption can lead to decreased folate absorption and increased oxidative stress through ethanol metabolism [49,50]. Nutrient compounds found abundantly in produce have been shown to reduce inflammation and oxidative stress, maintain proper function of cellular processes, and inhibit angiogenesis, theoretically defending high-risk individuals against the carcinogenic effects of tobacco and alcohol use[51,52]. Preclinical and clinical studies have previously elucidated several bioactive agents believed to affect cell-mediated immune response [53,54]. While the whole foods diet did not appear to significantly affect TILs in our cohort, we did find several significant associations between serum carotenoids and tocopherols on tumor immune response.

Serum carotenoids and tocopherols have been established by our group and others as valid biomarkers of fruit and vegetable intake [38,55]. Carotenoids, found abundantly in fruits and vegetables, represent a diverse group of natural polyene pigments and can be classified into xanthophylls (b-cryptoxanthin, lutein, and zeaxanthin) and carotenes (a-carotene, b-carotene, and lycopene) [56,57]. Both classes of carotenoids act as antioxidants, efficiently quenching singlet oxygen, reducing damage associated with reactive oxygen species and inhibiting lipid peroxidation [58-60]. In this study, we found significant decreases in the negative prognostic marker CD68+ associated with higher consumption of xanthophylls, lycopene and total carotenoids. These findings coincide with previous reports that higher pretreatment serum carotenoids are associated with progression-free survival in HNSCC patients[61], as well as the overall survival benefit associated with high serum xanthophyll[62] and lycopene concentrations [63]. Tocopherols, commonly known as vitamin E, have long been used clinically for a variety of oxidative stress-induced conditions[64] due to their functionality in trapping free radicals and protecting lipids in

the cell membranes from oxidation[65,66]. In our HNC cohort, we found increased levels of serum gamma-tocopherol to significantly increase CD8+ and FOXP3+ infiltrates in adjusted models. Gamma-tocopherol has previously been shown to be a stronger regulator of inflammation than alpha-tocopherol, namely through inhibition of cyclooxygenase (COX) and modulation of reactive oxygen species[67,68]. Several studies have also reported vitamin E supplementation to enhance CD4+ lymphocyte proliferation, we well as improve CD4+/CD8+ ratios[69,70]. Moreover, gamma-tocopherol, but not alpha-tocopherol, has recently been reported to exhibit anti-proliferative and pro-apoptotic effects selectively on cancer, but not normal epithelial cells [71].

Although limited, several studies have suggested an increased risk of HNSCC incidence and mortality associated with intake of red and processed meat, eggs and dairy [42,72-74]. In this study, the Western dietary pattern, characterized by a high consumption of red and processed meats, refined grains, potatoes, French fries, high-fat dairy, condiments, desserts, snacks and sugar-sweetened beverages, was found to decrease all TILs except CD68+, with significant decreases noted for CD8+ and FOXP3+. Furthermore, we found that among those individuals who had both dietary and carotenoid data, participants who consumed a high Western dietary pattern had significantly lower levels of beta carotene, xanthophylls, total carotenoids, as well as alpha-, gamma- and total tocopherols. These results suggest that study participants who consumed a high Western dietary pattern were not only consuming unhealthier food but were also eating many fewer fruits and vegetables than those in the low Western dietary pattern group, thereby foregoing the potential protective effects of these nutrients. High intake of red and preserved meats have previously been implicated in other cancers due to the production of genotoxic heterocyclic amines (HCA) and aromatic hydrocarbons (AH) during cooking, as well as nitrates and nitrites for preservation[75-77]. The International Agency for Research on Cancer (IARC) has classified the consumption of processed meat as “carcinogenic to humans” (Group 1), and red meat as “probably carcinogenic to humans” (Group 2A) through mechanisms linked to DNA damage and promotion of inflammation [78,79]. Based on the current evidence, it is reasonable to believe that there may be synergistic effects associated with tobacco, alcohol and meat consumption on HNSCC that warrant further investigation.

TILs are of particular importance in HNSCC due to the close proximity of these tumors to lymphoid tissue in the Waldeyer's ring, as well as the infectious etiology associated with oropharyngeal and nasopharyngeal carcinoma. Albeit being more aggressive, HPV and EBV related malignancies are associated with improved response to treatment, believed to be attributable to the presence of an increased immune response against the viral antigens. Nevertheless, irrespective of infection, TILs have consistently been shown to be an important prognostic tool in guiding HNSCC treatment. In a large retrospective study, increased CD4+ helper T and CD8+ cytotoxic/suppressor T cell levels significantly improved both overall and recurrence free survival in HNC patients; similar findings were reported between these infiltrates and oral cavity[80], laryngeal[81,82], oro- and hypopharyngeal[12,13,17] carcinoma, particularly among patients receiving chemotherapy. FOXP3+ regulatory T cells[15,83,84] , and more recently CD103+ infiltrates[40], or αEβ7 integrin, have also been shown to be positive prognostic markers in HNSCC, while CD68+ myeloid-derived suppressor cells have been associated with poor prognosis [15]. Accounting for the effect of diet on tumor immune response, we found that among those with high CD4+, CD68+, CD103+ and FOXP3+ markers, consumption of a high Western dietary pattern was associated with significantly worse overall survival when compared to those with a low Western dietary pattern; similar findings were noted between high CD4+ and FOXP3+ infiltrates and high Western dietary pattern on recurrence-free survival. These results are particularly interesting because previous studies conducted by our group did not find differential survival among those consuming various levels of the Western diet[25]. Given the reduction of all but CD68+ TILs found to be associated with increased Western dietary pattern, and the significant interaction terms between the aforementioned TILs and Western diet, it is reasonable to believe that the protective effect conferred by TILs may be modified by diet. In order to validate these results, future research should be conducted among HNSCC patients.

The results of this study should be interpreted in light of several limitations. Due to the fact that the FFQ was designed to assess dietary intake over the course of the year preceding HNC diagnosis, the effect of lifetime dietary exposure could not be assessed with respect to immune response and recall bias could have occurred. In addition, our sample size precluded us from being able to assess the associations of interest in stratified models, particularly evaluating differences in associations by disease site, smoking status or treatment modality. Nevertheless,

smoking and alcohol status were not found to be significant confounders when using backward selection, and sensitivity analyses integrating smoking and alcohol consumption into multivariate models did not alter the overall results of the study; treatment modality was found to be highly colinear with disease site, therefore only site was selected as a covariate in adjusted models. While TMAs are significantly more cost and time effective than staining and assessing entire slides, they do not fully capture the heterogeneity present within tumors, thereby limiting our ability to draw conclusions on the overall tumor microenvironment. In an attempt to minimize any potential confounding introduced by using TMAs, we adjusted the TIL counts by the percentage of tumor parenchyma present in each core, ran the samples in triplicate to best assess the overall sample, had a technician blinded to clinical status count the TILs and adjusted for batch effects in statistical models.

In conclusion, dietary patterns and serum carotenoids may play an important role in modifying TILs, and ultimately, outcome after diagnosis with HNSCC. As tumor immune response continues to emerge as an important prognostic tool in risk and treatment stratification, particularly with the advent of immunotherapy and personalized medicine, it is important to account for and educate patients on the role of modifiable lifestyle factors in these contexts. While further research is needed in order to confirm these results and elucidate some of the underlying biological mechanisms between Western dietary patterns, inflammation and immune response, the results of this study could inform dietary interventions among high risk individuals in an attempt to curb risk and improve HNSCC prognosis.

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Table 7.1a: Patient characteristics by dietary pattern

	Whole Foods Diet			Western Pattern		
	Low (N=116)	High (N=117)	P-value	Low (N=116)	High (N=117)	P-value
Age: Mean±SD [range], y	59±11 [25-95]	62±11 [39-92]	0.04	62±12 [30-95]	59±10 [25-92]	0.12
Sex, N (%)			0.25			0.02
Male	89 (79.72)	82 (70.09)		77 (66.38)	94 (80.34)	
Female	27 (23.28)	35 (29.91)		39 (33.62)	23 (19.66)	
Race			0.55			0.26
White	109 (93.97)	114 (97.44)		112 (96.55)	111 (94.87)	
Black	3 (2.59)	1 (0.85)		1 (0.86)	3 (2.56)	
American Indian	3 (2.59)	1 (0.85)		1 (0.86)	3 (2.56)	
Other	1 (0.86)	1 (0.85)		2 (1.72)	0 (0.00)	
Education			0.001			0.75
Less than High School	10 (8.70)	6 (5.13)		9 (7.83)	7 (5.98)	
High School/GED	46 (40.00)	25 (21.37)		36 (31.30)	35 (29.91)	
Some College	42 (36.52)	45 (38.46)		40 (34.78)	47 (40.17)	
4-yr Degree	10 (8.70)	15 (12.82)		11 (9.57)	14 (11.97)	
More than 4-yr Degree	7 (6.09)	26 (22.22)		19 (16.52)	14 (40.17)	
BMI: Mean±SD [range], kg/m^2	26.93±6.02 [16.01-47.94]	28.15±5.53 [16.44-54.36]	0.11	27.17±5.29 [16.01-40.21]	27.92±6.26 [16.02-54.36]	0.33
Stage, N (%)			0.92			0.96
1	16 (13.79)	19 (16.24)		18 (15.52)	17 (14.53)	
2	15 (12.93)	17 (14.53)		17 (14.66)	15 (12.82)	
3	18 (15.52)	18 (15.38)		17 (14.66)	19 (16.24)	
4	67 (57.76)	63 (53.85)		64 (55.17)	66 (56.41)	
Site, N (%)			0.95			0.08
Larynx	21 (18.10)	22 (18.80)		14 (12.07)	29 (24.79)	
Oral Cavity	50 (43.10)	53 (45.30)		55 (47.41)	48 (41.03)	
Oropharynx	42 (36.21)	40 (34.19)		45 (38.79)	37 (31.62)	
Hypopharynx	3 (2.59)	2 (1.71)		2 (1.72)	3 (2.56)	
HPV Status, N (%)*			0.56			0.53
Positive	40 (34.48)	33 (28.21)		49 (42.24)	58 (49.57)	
Negative	50 (43.10)	57 (48.72)		39 (33.62)	34 (29.06)	
Invalid/Missing	26 (22.41)	27 (23.08)		28 (24.14)	25 (21.37)	
ACE-27 Score, N (%)			0.70			0.50
None	33 (28.70)	32 (27.35)		27 (23.48)	38 (32.48)	
Mild	59 (51.30)	56 (47.86)		60 (52.17)	55 (47.01)	
Moderate	16 (13.91)	17 (14.53)		18 (15.65)	15 (12.82)	
Severe	7 (6.09)	12 (10.26)		10 (8.70)	9 (7.69)	
Drinking Status, N (%)			0.87			0.16
Never	9 (7.76)	9 (7.69)		11 (9.48)	7 (5.98)	
Current	80 (68.97)	84 (71.79)		75 (64.66)	89 (76.07)	
Former (quit >12 months)	27 (23.28)	24 (20.51)		30 (25.86)	21 (17.95)	
Smoking, N (%)			0.01			0.06
Never	30 (25.86)	27 (23.08)		36 (31.03)	21 (17.95)	
Current	55 (47.41)	38 (32.48)		41 (35.34)	52 (44.44)	
Former (quit >12 months)	31 (26.72)	52 (44.44)		39 (33.62)	44 (37.61)	
Treatment Modality, N (%)			0.90			0.86
Surgery Alone	28 (24.14)	30 (25.64)		29 (25.00)	29 (24.79)	
Surgery + Adj rad	17 (14.66)	19 (16.24)		16 (13.79)	20 (17.09)	
Surgery + Adj chemorad	14 (12.07)	14 (11.97)		16 (13.79)	12 (10.26)	
Radiation Alone	10 (8.62)	6 (5.13)		7 (6.03)	9 (7.69)	
Chemorad Alone	38 (32.76)	38 (32.48)		40 (34.48)	36 (30.77)	
Chemo Alone	1 (0.86)	3 (2.56)		1 (0.86)	3 (2.56)	
Palliative, Unknown	8 (6.90)	7 (5.98)		7 (6.03)	8 (6.84)	
Deceased, N (%)	33 (28.45)	32 (27.35)	0.85	30 (25.86)	35 (29.91)	0.49

* one patient missing ACE-27 score

Abbreviations: ACE-27, Adult Comorbidity Evaluation-27; BMI, body mass index; HPV, human papillomavirus; SD, standard deviation.

Table 7.1b. Serum micronutrient concentrations by dietary pattern

	Whole Foods Diet			Western Pattern		
	Low (N=34)	High (N=36)	P-value	Low (N=25)	High (N=45)	P-value
Serum Micronutrients µg/d, Mean (SD)						
Alpha Carotene	0.01 (0.02)	0.02 (0.01)	0.15	0.02 (0.01)	0.01 (0.02)	0.49
Beta Carotene	0.05 (0.07)	0.07 (0.10)	0.03	0.08 (0.11)	0.04 (0.05)	0.0002
Xanthophylls	0.14 (0.10)	0.17 (0.13)	0.13	0.18 (0.14)	0.14 (0.09)	0.02
Lycopene	0.05 (0.04)	0.05 (0.04)	0.55	0.05 (0.03)	0.05 (0.04)	0.25
Total Carotenoids	0.26 (0.14)	0.30 (0.24)	0.01	0.34 (0.27)	0.25 (0.14)	0.001
Alpha tocopherol	7.98 (5.02)	10.33 (6.07)	0.29	10.59 (7.49)	8.34 (4.13)	0.001
Gamma tocopherol	1.14 (0.57)	1.18 (0.72)	0.22	1.18 (0.55)	1.13 (0.79)	0.04
Total tocopherol	9.12 (5.32)	11.50 (6.12)	0.44	11.72 (7.76)	9.52 (4.22)	0.001

Table 7.2: Linear Regression Model Assessing the Association between Tumor Infiltrating Lymphocytes and Binary Dietary Pattern

	Tumor Infiltrating Lymphocytes (TILs)									
	Unadjusted					Adjusted*				
	CD4	CD8	CD68	CD103	FOXP3	CD4	CD8	CD68	CD103	FOXP3
Sample Size	228	228	130	93	232	228	228	130	93	232
Whole Foods Pattern β(SE)										
≤ -0.20	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
> -0.20	-1.47 (6.65)	4.03 (9.13)	0.86 (4.78)	19.84 (21.90)	3.39 (5.37)	-1.89 (6.71)	3.48 (9.26)	1.54 (4.92)	20.82 (22.00)	4.54 (5.42)
p-value for trend	0.82	0.66	0.86	0.37	0.53	0.78	0.71	0.76	0.35	0.40
Western Pattern β(SE)										
≤ -0.16	ref	**ref**	ref	ref	**ref**	ref	**ref**	ref	ref	**ref**
> -0.16	-9.00 (6.63)	**-19.99 (9.04)**	0.28 (4.78)	-35.88 (22.00)	**-10.78 (5.33)**	-8.52 (6.76)	**-19.99 (9.27)**	-0.13 (4.82)	-41.72 (23.16)	**-12.54 (5.42)**
p-value for trend	0.18	**0.03**	0.95	0.11	**0.04**	0.21	**0.03**	0.98	0.08	**0.02**
*** Adjusted for age, sex and TMA**										

Table 7.3: Logistic Regression Analysis Assessing the Association between Tumor Infiltrating Lymphocytes and Serum Carotenoids

	Tumor Infiltrating Lymphocytes (TILs)							
	Unadjusted				Adjusted‡			
	CD4	CD8	CD68	FOXP3	CD4	CD8	CD68	FOXP3
Sample Size	69	69	68	70	69	69	68	70
Alpha Carotene	OR (CI)	OR (CI)	OR (CI)	OR (CI)	OR (CI)	OR (CI)	OR (CI)	OR (CI)
Low	(Ref)	**(Ref)**	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
High	1.48 (1.01, 2.18)	**2.25 (1.53, 3.30)**	0.86 (0.59, 1.25)	1.54 (1.05, 2.25)	1.05 (0.64, 1.70)	1.05 (0.65, 1.71)	0.53 (0.33, 0.85)	0.44 (0.25, 0.79)
p-value for trend	0.05	**<.0001**	0.42	0.03	0.85	0.84	0.01	0.01
Beta Carotene								
Low	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
High	0.91 (0.62, 1.34)	1.51 (1.03, 2.20)	1.08 (0.74, 1.57)	1.13 (0.78, 1.65)	0.74 (0.47, 1.17)	0.95 (0.59, 1.53)	0.91 (0.58, 1.40)	0.48 (0.29, 0.82)
p-value for trend	0.64	0.03	0.70	0.52	0.19	0.83	0.66	0.01
Xanthophylls								
Low	(Ref)	(Ref)	**(Ref)**	(Ref)	(Ref)	(Ref)	**(Ref)**	(Ref)
High	1.17 (0.80, 1.71)	0.98 (0.67, 1.43)	**0.21 (0.14, 0.32)**	0.93 (0.64, 1.36)	0.73 (0.45, 1.18)	0.58 (0.35, 0.96)	**0.12 (0.07, 0.21)**	0.91 (0.55, 1.51)
p-value for trend	0.43	0.92	**<.0001**	0.72	0.20	0.03	**<.0001**	0.71
Lycopene								
Low	(Ref)	(Ref)	**(Ref)**	(Ref)	(Ref)	(Ref)	**(Ref)**	(Ref)
High	0.83 (0.56, 1.21)	1.51 (1.03, 2.20)	**0.53 (0.36, 0.78)**	1.18 (0.81, 1.73)	0.70 (0.43, 1.14)	0.47 (0.27, 0.81)	**0.36 (0.22, 0.61)**	1.02 (0.59, 1.75)
p-value for trend	0.33	0.03	**0.001**	0.38	0.15	0.01	**0.0001**	0.95
Total*								
Low	(Ref)	(Ref)	**(Ref)**	(Ref)	(Ref)	(Ref)	**(Ref)**	(Ref)
High	0.90 (0.61, 1.32)	1.23 (0.85, 1.80)	**0.46 (0.31, 0.67)**	0.80 (0.55, 1.17)	0.61 (0.37, 1.02)	0.44 (0.26, 0.75)	**0.31 (0.19, 0.51)**	0.56 (0.32, 0.97)
p-value for trend	0.57	0.27	**<.0001**	0.25	0.06	0.003	**<.0001**	0.04
Alpha tocopherol								
Low	**(Ref)**	**(Ref)**	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
High	**2.34 (1.59, 3.46)**	**2.09 (1.43, 3.06)**	1.30 (0.89, 1.89)	1.68 (1.15, 2.44)	1.61 (1.00, 2.60)	1.68 (1.04, 2.71)	0.94 (0.60, 1.47)	1.08 (0.65, 1.80)
p-value for trend	**<.0001**	**0.0001**	0.17	0.01	0.05	0.03	0.78	0.77
Gamma tocopherol								
Low	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	**(Ref)**	(Ref)	**(Ref)**
High	0.69 (0.47, 1.02)	1.49 (1.02, 2.17)	1.54 (1.05, 2.24)	1.45 (1.00, 2.11)	0.73 (0.46, 1.15)	**2.21 (1.37, 3.56)**	1.89 (1.22, 2.93)	**4.26 (2.40, 7.58)**
p-value for trend	0.06	0.04	0.03	0.05	0.18	**0.001**	0.004	**<.0001**
Total tocopherol¥								
Low	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
High	1.79 (1.22, 2.64)	1.60 (1.10, 2.33)	1.12 (0.77, 1.62)	1.29 (0.89, 1.88)	1.25 (0.78, 1.99)	1.44 (0.90, 2.30)	0.96 (0.62, 1.49)	0.93 (0.56, 1.54)
p-value for trend	0.003	0.01	0.56	0.18	0.35	0.13	0.86	0.78

*** calculates as: alpha carotene+beta carotene+xanthophylls+lycopene**
¥ calculated as: alpha tocopherol+gamma tocopherol
‡ Age, sex, disease site and TMA adjusted

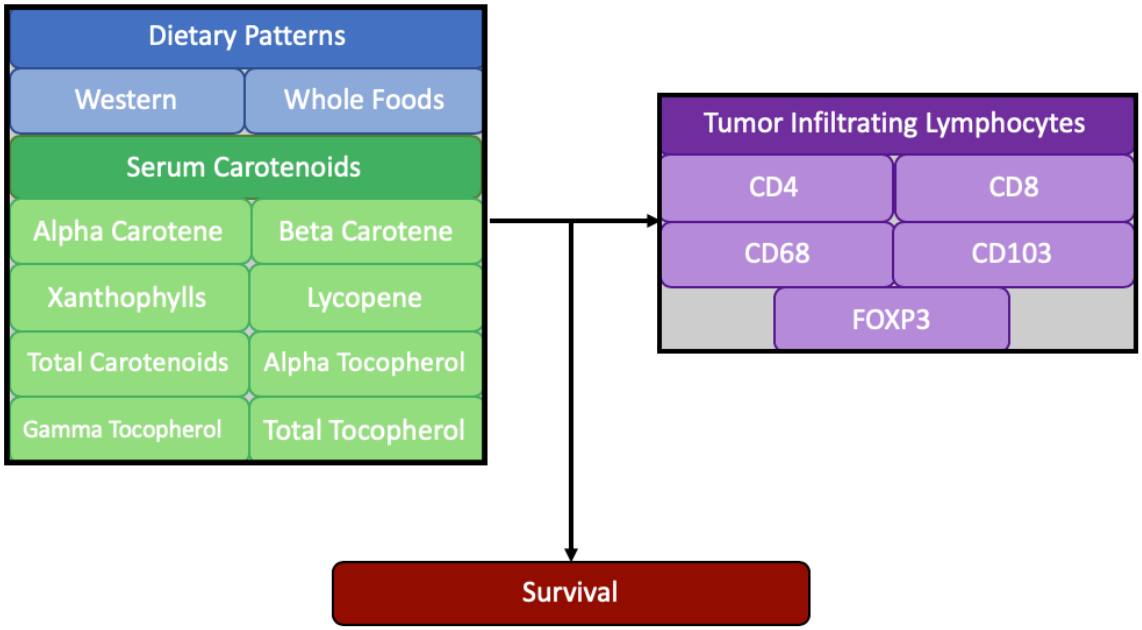
Table 7.4: Cox Proportional Hazard Models Assessing the Role of Tumor Infiltrating Lymphocytes and Diet on Recurrence Free Survival

	CD4		CD8		CD68		CD103		FOXP3	
	Low	High	Low	High	Low	High	Low	High	Low	High
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Whole Foods Pattern										
Low	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
High	1.31 (0.80, 2.15)	0.91 (0.49, 1.69)	0.97 (0.60, 1.58)	1.11 (0.60, 2.07)	1.32 (0.65, 2.65)	0.87 (0.36, 2.06)	1.76 (0.86, 3.57)	0.68 (0.22, 2.10)	1.61 (0.97, 2.68)	0.74 (0.41, 1.35)
p-value for interaction	0.37		0.73		0.45		0.17		0.06	
Western Pattern										
Low	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
High	0.58 (0.34, 0.99)	3.36 (1.73, 6.54)	1.08 (0.65, 1.78)	1.63 (0.86, 3.08)	1.32 (0.60, 2.92)	3.18 (1.19, 8.50)	0.59 (0.28, 1.24)	2.19 (0.67, 7.15)	0.62 (0.37, 1.05)	2.93 (1.56, 5.51)
p-value for interaction	**<.0001**		0.30		0.16		0.06		**0.0002**	
***Models adjusted for age, sex, stage, disease site, HPV status and TMA**										

Table 7.5: Cox Proportional Hazard Models Assessing the Role of Tumor Infiltrating Lymphocytes and Diet on Overall Survival

	CD4		CD8		CD68		CD103		FOXP3	
	Low	High	Low	High	Low	High	Low	High	Low	High
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Whole Foods Pattern										
Low	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
High	0.71 (0.43, 1.15)	1.72 (0.95, 3.11)	0.71 (0.43, 1.14)	1.15 (0.62, 2.11)	1.03 (0.51, 2.05)	0.64 (0.31, 1.31)	1.13 (0.45, 2.83)	1.09 (0.34, 3.56)	0.98 (0.61, 1.58)	1.02 (0.59, 1.79)
p-value for interaction	**0.02**		0.21		0.37		0.97		0.91	
Western Pattern										
Low	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
High	0.76 (0.46, 1.26)	1.77 (0.98, 3.17)	1.17 (0.73, 1.86)	1.21 (0.66, 2.23)	0.53 (0.25, 1.12)	2.42 (1.16, 5.06)	0.65 (0.27, 1.55)	3.64 (1.05, 12.64)	0.84 (0.52, 1.36)	3.09 (1.00, 3.11)
p-value for interaction	**0.03**		0.92		**0.004**		**0.03**		**0.05**	
***Models adjusted for age, sex, stage, disease site, HPV status and TMA**										

Figure 7.1: Conceptual Framework



Chapter 8

Conclusions

Cancer is one of the most common causes of both morbidity and mortality in the world today; more than 10 million new cases and 6 million deaths are attributed to cancer each year [1]. An estimated 20 million people worldwide are thought to be living with a cancer diagnosis, of which more than half reside in developing countries. If trends continue as they are, projections for 2020 show annual increases in both incidence and mortality rates of 15 million and 10 million respectively [2]. While a portion of these trends can be attributed to the aging global population, increasing prevalence of exposure to cancer risk factors in both high-income, and particularly in low- and middle- income countries, has unquestionably had an impact in driving chronic disease, and especially cancer rates [3].

This dissertation has built upon the body of literature that recognizes the importance of understanding head and neck cancer etiology from both preventative and treatment standpoints. We still lack knowledge and comprehensive understanding of the wide variety of environmental exposures that lead to HNSCC carcinogenesis, the role they play in cancer biology and the impact they have on global prevalence and survival trends. Head and neck cancer is chronically underfunded and understudied, receiving the second-fewest incidence-normalized research dollars of any common cancer [4]. In order for the scientific community to be better informed and adequately prepared to manage the increasing burden of disease, head and neck cancer epidemiology, and corresponding risk factors, must be better understood in order to improve cancer prevention, screening and treatment strategies.

Aim one, comprised of four manuscripts, leverages cancer registry data in order to assess trends in head and neck cancer incidence and mortality. The creation of registries is a crucial first step in developing and accessing strategies for both cancer prevention and patient care, consequently informing public health and clinical decisions on a local, and potentially global, scale. While

these studies are descriptive in nature, understanding temporal patterns in cancer trends is important in generating hypotheses regarding etiological changes that propel mechanistic studies that inform prevention and treatment modalities.

The first two papers focused specifically on nasopharyngeal carcinoma, which is analyzed separately from the other head and neck cancer subsites due to its unique etiology and geographical distribution. Thailand is considered an endemic country for NPC, whereas the United States has historically reported very few cases, resulting in limited studies with small sample sizes. Nasopharyngeal carcinoma is by far not the only cancer type to exhibit varying incidence across countries. While some of these variabilities may be due to genetic factors, the vast majority of cancers, NPC included, do not display hallmarks of simple inheritance[5]. Migration studies, examining the role of population-based relocation on cancer rates, have long been used to illustrate the effects of environmental and lifestyle changes conferred by the host country on the incidence of cancer. A seminal study on the topic tracked Japanese immigrants to Hawaii and California, discovering that cancer incidence shifts with successive generations to more closely mimic trends apparent among their white peers [6]. Manuscript one of this aim investigated incidence and mortality trends in NPC between 1990 and 2014 across four Thai registries and compared them to those found among Asian/Pacific Islanders (A/PI) in the US. To our surprise, US Asian/Pacific Islanders had higher age-standardized rates of NPC than the endemic Thai population. Furthermore, the A/PI displayed a higher incidence of the non-keratinizing subtype, which has been reported to account for ~95% of cases in endemic regions[7] and predominated among cases diagnosed in Chiang Mai, Khon Kaen and Lampang. Due to data limitations within SEER to ascertain Thai ethnicity and generational shift, a true migration study could not be performed, but the findings still emphasized an interesting phenomenon that highlights the importance of further etiological studies into NPC to determine the genetic and environmental drivers of this cancer. Additional results from this paper found the keratinizing NPC subtype, typically associated with combustible tobacco use, to be decreasing among Chiang Mai males, as well as both sexes in Songkhla and the US; these reductions in incidence correspond to reports of decreased tobacco use. Finally, survival analyses underscored the successes of chemoradiation treatment for non-keratinizing NPC, as well as demonstrated the

continuing challenges faced by rural populations to gain access to care, irrespective of the introduction of universal health care in Thailand.

The second paper in this aim more closely investigated NPC incidence and mortality trends by histologic subtype, race, sex and age in the US. While overall NPC appeared to be decreasing across all strata, much of this effect was due to reductions in keratinizing NPC, again corresponding to decreased smoking rates across the US. Trends in the non-keratinizing subtype, on the other hand, raised some interesting questions regarding the etiological differences between differentiated and undifferentiated tumors. The current state of literature suggests that all non-keratinizing nasopharyngeal carcinomas are associated with EBV infection, but this study found stark differences in trends between these two subtypes. Undifferentiated lesions were shown to be consistently decreasing, while the differentiated tumors increased across all strata, reaching rates are high as 4% annually among white males and females, as well as black males. While unable to confirm etiology using these data, exploratory analyses into NK/T-cell lymphoma, another EBV related cancer, showed similar increasing incidence rates as NPC, further strengthening the hypothesis that EBV may be a relevant factor in explaining the observed rates. As expected based on the previously mentioned paper, Asian/Pacific Islanders had rates that were more than five times higher among females and seven times higher among males when compared with their white counterparts. This race group also appeared to experience the highest 5-year relative survival estimates, while black males displayed the worst survival regardless of histology. African Americans also appeared to exhibit a bimodal distribution in cases, with 25% of patients being diagnosed before age 40 years compared with 12% of their white counterparts. This disparity was particularly pronounced in the nonkeratinizing subtype, where 32% of cases among blacks occurred before the age of 40. Previous studies using NHANES have reported significantly higher EBV seroprevalence among black children as opposed to their white peers[8 9], raising interest in whether earlier life exposure to EBV may increase the risk of NPC development at younger ages. Overall, this paper stressed the need for further research into NPC etiology across race and histological subtype. Due to the fact that US-based prospective cohort studies with comprehensive exposure assessment would require decades to recruit a sufficient sample size to assess gene-environment interactions, multicenter

studies or retrospective collection of tumor tissues would be needed to better understand the nuances of this cancer in the United States.

The remaining two papers of aim one both assessed overall and subsite specific head and neck cancer trends. The first used data from IARC's CI5*plus* database from three Thai registries (Songkhla, Chiang Mai, Lampang), Singapore, Manila, Shanghai and compared them to rates seen in the US. Overall rates of head and neck cancer appeared to decrease among all the groupings except Shanghai males. Further subsite analyses revealed that laryngeal cancer, the anatomical site most strongly associated with combustible tobacco use, has been increasing at an annual percent change of 9.1 from 1988 to 2002 in Shanghai. As the world's largest tobacco manufacturer, China accounts for about 30% of the world's cigarette consumption, and other studies have previously reported minimal interest in smoking cessation within this population[10]. Although the overall rates in Shanghai still appeared to be much lower than expected, given the high uptake in tobacco use, smoking-related illnesses should be closely monitored within this population and stronger regulations should be considered.

Khon Kaen, Thailand is one of the only places worldwide with higher reported incidence of head and neck cancer among women than men [11]. This phenomenon is particularly striking for oral cavity cancer, where ~74% of cases are diagnosed among women. The custom of chewing betel quid is highly prevalent among the older generation of women living in Northeastern Thailand, and this etiological factor accounts for disproportionately high cancer rates in the region. Years of educational and political campaigns have reduced this high-risk behavior, especially among the younger generation, leading to observed decreases in oral cavity cancer at an annual percent change of 4.6; nevertheless, tongue and pharyngeal carcinomas appear to be increasing in Thailand. In the Western world, changes in cultural norms and improved control over combustible tobacco use has elucidated the role of high-risk HPV as an important etiological factor in oral, and especially oropharyngeal, carcinoma. This subset of the disease disproportionately effects younger individuals who may not otherwise display high risk behaviors for HNSCC development. However, to date, there have been no studies published on HPV prevalence in HNSCC in Southeast Asia. Given the findings of increased HPV prevalence in the North America and Europe, it is reasonable to believe that the observed increases in

tongue and pharyngeal carcinoma noted in Thailand may similarly be due to HPV integration. Although believed to be more aggressive, HPV positive tumors have been shown to be more responsive to treatment, leading the American Joint Committee on Cancer to suggest restaging these lesions[12]; this suggestion had led to a de-escalation of treatment, consequently improving morbidity and mortality among afflicted patients. If the etiological shift is confirmed in Thailand, similar alteration to screening and treatment can be adopted, which would improve quality of life, and hopefully survival statistics for this cancer subtype.

The objective of aim two was to validate the finding from the previous manuscript using molecular epidemiology. 107 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from patients seen at Srinagarind Hospital in Khon Kaen, Thailand between 2012 and 2017 were obtained, alongside corresponding patient data, and sent to the University of Michigan for analysis. The samples were tested for the presence of HPV using polymerase chain reaction and p16 staining. After exclusion of 11 patients due to oral cavity, rather than oropharyngeal, carcinoma diagnosis 14 patients were determined to be infected with HPV16, 2 with HPV18 and 1 was co-infected with both HPV16 and HPV18. On average, HPV+ patients were younger and more likely to have tonsil cancer. Taken together, these characteristics, in addition to the clear survival benefit shown to be conferred by the presence of HPV in our study, not only corroborated the suggested findings of the aforementioned descriptive epidemiology study, but also supported the adaptation of p16 staining and consequent restaging of oropharyngeal carcinomas as suggested by the American Joint Committee on Cancer. While these results should be validated across other parts of Southeast Asia, implementation of these protocols would allow for improved monitoring and more informed treatment decisions. Being the first study to show the existence, and prognostic significance, of HPV in the region, our hopes are that these results will inform medical practice and improve quality of life as well as survival outcomes among afflicted patients.

The final aim utilized data from the University of Michigan Head and Neck Cancer SPORE to evaluate the potential role of diet on tumor immune response and, ultimately, outcome. On a molecular level, the literature into the effects of diet on head and neck cancer is surprisingly limited. Individually, TILs have been shown to be important indicators in determining treatment

efficacy among HNSCC patients, while diet has been demonstrated to have a bi-directional effect on head and neck risk, progression and prognosis. The use of diet to modulate immune system function by mediating inflammation within the tumor microenvironment has been proposed for HNSCC, nevertheless, no studies to date have tested this hypothesis. In this study, we found the Western dietary pattern, characterized by high consumption of red and processed meats, refined grains, potatoes, French fries, high-fat dairy, condiments, desserts, snacks and sugar-sweetened beverages, to significantly decrease the favorable CD8+ and FOXP3+ markers. While high levels of serum gamma tocopherol were shown to increase these markers, elevated levels of xanthophylls, lycopene and total carotenoids appeared to decrease the deleterious CD68+ TIL count. Survival analyses assessing the joint association between TILs and dietary pattern demonstrated that among those with high CD4+, CD68+, CD103+ and FOXP3+ markers, having a high Western dietary pattern significantly increased the risk of overall mortality when compared to those with a low Western dietary pattern. Similar results were found for recurrence-free survival, CD4+, FOXP3+ and Western dietary pattern. While the results of this study need to be validated, and further studies into the underlying mechanisms of this association need to be elucidated, the findings reported here can be used as a support to inform dietary interventions among high risk patients. As the medical world continues to gain interest in the utility of immunotherapy and personalized medicine, and screening for molecular markers such as TILs becomes more routine, it is important to be able to inform patients on modifiable behaviors that may improve their chances of a favorable outcome.

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