RESEARCH ARTICLE

Editorial Process: Submission:11/10/2023 Acceptance:02/20/2024

Head and Neck Cancer of Unknown Primary: A Multicenter Retrospective Cohort study in Northern Thailand, an Endemic Nasopharyngeal Cancer Area

Pichit Sittitrai[1], Donyarat Ruenmarkkaew[1]*, Imjai Chitapanarux[2], Pooriwat Muangwong[2], Kanchana Kangsadarnwiroon[3], Ratcharin Benjawongsatien[4], Chonticha Srivanitchapoom[5], Supawit Donchalermpak[6], Tussawan Asakit[7]

Abstract

Objective: This study aimed to evaluate the characteristics and oncological outcomes of head and neck carcinoma of unknown primary (HNCUP) patients in an endemic nasopharyngeal cancer (NPC) area. **Methods:** One hundred and forty-four HNCUP patients curatively treated between January 1995 and December 2022 from 5 centers were retrospectively recruited onto the study to analyze the clinicopathological characteristics and oncological outcomes and compare them with historical data. A multivariate Cox proportional hazards model analysis was performed to evaluate factors affecting survival outcomes. A propensity-matched pair analysis of the patients with positive and negative EBV-encoded small RNA (EBER) staining was applied to compare the characteristics and outcomes between the two groups. **Results:** The median follow-up time was 45 months. Most patients (88.2%) received total mucosal irradiation (TMI). Primary tumor emergence (PTE) was detected in 6 patients (4.2%) who did not have TMI. The 5-year overall survival (OS), disease-free survival, and locoregional recurrence-free survival were 51.3%, 64.9%, and 72.7%, respectively. Extranodal extension and N3 compared with the N1 stage were the significant independent predictors for OS (HR 2.90, 95% CI 1.12-7.51, p = 0.028 and HR 3.66, 95%CI 1.23-11.89, p = 0.031, respectively). The matched-pair analysis demonstrated comparable all survival outcomes between the EBER-positive and -negative groups. All patients in the matched pair analysis received TMI, and no PTE was detected. **Conclusion:** Our survival outcomes were comparable to previous studies with a low rate of PTE. The matched pair analysis of EBER-positive and -negative groups revealed similar oncological outcomes and no primary tumor emergence when total mucosal irradiation was administered.

Keywords: Unknown primary tumor- squamous cell carcinoma- head and neck cancer- nasopharyngeal cancer

Asian Pac J Cancer Prev, **25 (2)**, 699-708

Introduction

Carcinoma of unknown primary (CUP) is defined as a histological diagnosis of metastases without diagnosing a primary tumor [1]. Cases account for approximately 3-5% of all malignant neoplasms [2, 1]. In addition to lung, liver, and bone, the most frequent localizations of the metastases are cervical lymph nodes, known as head and neck cancer of unknown primary (HNCUP) [1]. Up to 10% of all cervical lymph node metastases present without a known primary site and are responsible for 2-4% of head and neck carcinoma patients [3, 2]. The most common histology encountered in HNCUP patients is squamous cell carcinoma in 65-76% of cases, followed by undifferentiated carcinoma (14%), adenocarcinoma (13%), and nasopharyngeal-type undifferentiated carcinoma (8%) [4-6]. The lymph node metastases are usually localized in the upper two-thirds of the neck (levels I-III), mainly deriving from squamous cell carcinoma of the head and neck [7, 8]. Metastases localized in the lower third of the neck (levels IV-V) suggest a primary tumor located under the clavicle, usually an adenocarcinoma [9, 8]. Lymph node metastases commonly occur unilaterally; bilateral metastases are only observed in about 10% of cases [3, 10].

The initial evaluation of HNCUP includes medical

*[1]Department of Otolaryngology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. [2]Division of Radiation Oncology, Department of Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. [3]Department of Otolaryngology, Buddhachinaraj Hospital, Pitsanulok, Thailand. [4]Department of Radiation Oncology, Buddhachinaraj Hospital, Pitsanulok, Thailand. [5]Department of Otolaryngology, Phayao Hospital, Phayao, Thailand. [6]Department of Otolaryngology, Lampang Hospital, Lampang, Thailand. [7]Department of Radiation Oncology, Lampang Cancer Hospital, Lampang, Thailand. *For Correspondence: rdonyarat@gmail.com*

history, complete ear, nose, and throat examination, and flexible nasopharyngolaryngoscopy. A lymph node fine needle aspiration biopsy (FNAB) is done to determine the primary site [4, 1]. When histological confirmation of squamous cell carcinoma is revealed, diagnostic studies of HPV/p16 and EBV status are recommended [11]. In addition, a computed tomographic (CT) or magnetic resonance imaging (MRI) scan of the head and neck followed by panendoscopy with guided biopsy of suspected mucosa and palatine tonsillectomy can be used and detect the primary tumor in up to 40% of cases [12]. Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) can help to locate the primary site in a minority of patients [13, 14]. More recently, in Western countries, transoral robotic lingual tonsillectomy has emerged as another tool to assist in the diagnosis of HNCUP and may increase the primary detection rate by approximately 20% [15, 16].

When all diagnostic evaluations have been unable to detect a primary site, a final diagnosis of carcinoma of unknown primary is made [11]. Several theories have attempted to explain the existence of HNCUP, including that the primary site may undergo regression or destruction, the primary site may be undetectable due to location, or the primary site may be undetectable due to a very small size [17].

Treatment for HNCUP is focused on controlling the disease in the neck and preventing the emergence of primary cancer [11]. However, HNCUP is a rare disease inhibiting the carrying out of prospective multicenter, randomized trials; hence, consensus on optimal treatment has yet to be established. Treatment recommendation is, therefore, usually based on results from retrospective studies and institutional preference. Surgery or radiotherapy alone is considered for early nodal stages of the disease, and a combination of surgery followed by adjuvant (chemo)radiotherapy or primary chemoradiotherapy with or without surgery is required for more advanced disease [18].

This multicenter study aimed to analyze the clinicopathological characteristics, oncological outcomes, and prognostic factors, particularly pertaining to the status of the Ebstein-Barr virus (EBV) of the metastatic squamous cell and undifferentiated carcinomas to cervical lymph nodes from an unknown primary in our region, an endemic nasopharyngeal cancer (NPC) area [19, 20]. We also aimed to compare the results with studies from other endemic NPC cancer countries and non-endemic NPC countries.

Materials and Methods

Data was collected from patients with carcinoma metastatic to cervical lymph nodes without identifiable primary tumor at the start of treatment at the Departments of Otolaryngology as well as Radiotherapy and Radiation Oncology of 5 referral centers, including Maharaj Nakorn Chiang Mai Hospital, Buddhachinaraj Hospital, Lampang Hospital, Lampang Cancer Hospital, and Phayao Hospital. The data was collected between January 1995 and December 2021, with follow-up recorded up to December 2022, and retrospectively analyzed. This study did not include patients with histopathological diagnoses other than squamous cell or undifferentiated carcinomas, previously or palliatively treated, or with distant metastasis.

All procedures contributing to this work complied with the ethical standards of the relevant national and institutional guidelines on human experimentation and with the Declaration of Helsinki 1975, revised in 2013. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, and all the participating centers. The reference number is 398/2021. As patient identities were anonymized, the requirement for informed consent was waived by the Ethics Committees.

Diagnostic workup

All patients were classified or reclassified in accordance with the American Joint Committee for Cancer Staging classification, 8th edition [21]. After a thorough head and neck examination, fine needle aspiration or incisional/excisional lymph node biopsy was performed. Next, computed tomography (CT) scan, magnetic resonance imaging (MRI), and abdominal sonography were done to evaluate the metastatic lymph node and to identify the primary tumor and distant metastasis. Due to the high cost of FDG-PET/CT scan, this imaging was limited to 15 patients during the study period. However, one patient with an increased FDG uptake at the base of the tongue and having a positive biopsy result of mucoepidermoid carcinoma was excluded. Therefore, the remaining 14 patients were included in the study. Following the imaging studies, the patients received an examination under general anesthesia through panendoscopy (nasopharyngoscopy, laryngoscopy, tracheobronchoscopy, and esophagoscopy), manual palpation of the oral cavity and oropharynx, and guided biopsy of the area of suspicion. Ipsilateral or bilateral palatine tonsillectomy was performed in 91.7% of the patients during the study period, and primary tumors were detected in 8.5%. This group of patients with identified primaries were excluded from the study. In cases where EBV-encoded small RNA (EBER) detection by in situ hybridization from lymph node biopsy was done, the nasopharynx would be carefully evaluated, and a nasopharyngeal biopsy would also be performed. Due to the low incidence of HPV-related oropharyngeal carcinoma in this area, limited cases of p16 immunohistochemical staining were completed and not included in the analysis. However, lingual tonsillectomy would be performed in patients with p16-positive lymph nodes with no identifiable primary tumors at palatine tonsils.

Treatment

Treatment planning was done following a multidisciplinary discussion with the patient's preferences, comorbidities, and performance status, including surgery with or without (chemo)radiotherapy and radiotherapy with or without chemotherapy being considered.

Surgery, as either a primary or a salvage treatment, included comprehensive neck dissection (levels I-V),

unilaterally or bilaterally, according to clinical and the CT scan or MRI findings.

Radiotherapy consisted of 2 Gray (Gy) per fraction, 5 fractions per week. The radiation doses of definite, adjuvant, and prophylactic radiotherapy were 66 to 72 Gy, 60 to 66 Gy, and 50-54 Gy, respectively. The radiation volume could be 1) total mucosal irradiation (TMI), including the potential mucosal primaries (bilateral nasopharynx, oral cavity, oropharynx, hypopharynx, and larynx), and bilateral neck, 2) limited mucosal irradiation (oropharynx and hypopharynx) and bilateral neck, or 3) unilateral or bilateral neck irradiation only.

The chemotherapy, usually administered in N2b (with multilevel lymph node) cases or those with higher-staged neck disease, consisted of tri-weekly induction with either cisplatin at 100 mg/m^2 and 5-fluorouracil at 400 mg/m^2 for 5 days or carboplatin AUC 2 and paclitaxel 50 mg/m^2. Concurrent chemoradiotherapy as a definitive or adjuvant treatment was administered with tri-weekly cisplatin at 100 mg/m^2 or carboplatin AUC 2.

Follow-up

After completion of treatment, follow-up protocols included clinical evaluation every 1-3 months in the first year, every 2-6 months in the second year, every 4-8 months in the third to fifth years, and every 12 months after the fifth year. In addition, a CT scan or MRI was performed at 8-12 weeks or FDG-PET/CT at 12-16 weeks after treatment, and then a CT scan was performed annually for 2 years and as indicated afterward.

Outcomes

The primary outcomes were overall survival (OS), disease-free survival (DFS), and locoregional recurrence-free survival (LRRFS). OS was defined as the time from starting treatment until the date of death from any cause. DFS was defined as the time from starting treatment until the date of first recurrence, independently of whether it was a mucosal tumor emergence, neck recurrence, or distant recurrence. LRRFS was defined as the time from starting treatment until the date of first mucosal tumor emergence or neck recurrence. In case of no death or disease recurrence, the observation was censored at the last follow-up visit.

The secondary outcome was to investigate the prognostic factors, particularly EBV status, on oncological outcomes.

Statistical analysis

Categorical variables were summarized as frequencies (%). Continuous variables were checked for normality and summarized as mean ± standard deviation if normally distributed or as median (interquartile range) if not. Pearson's chi-square or Fisher exact tests were used to compare categorical variables with frequencies more significant than 5 or smaller than 5, respectively. A Student's t-test was used to compare continuous variables which were normally distributed, and the Mann-Whitney U test was used for comparing continuous variables with a non-normal distribution. The Kaplan-Meier curve was used to analyze the survival data, and differences between groups were conducted using the log-rank test. Multivariate Cox proportional hazards model analyses were carried out to study the influence of clinical and pathological parameters on survival outcomes. Propensity score-matched analysis by logistic regression and 1:1 matching was performed based on the propensity score of each patient. The quality of the match was assessed by recalculating the standardized mean difference of each variable in the matched sample until a balance was achieved. A p-value less than 0.05 was considered statistically significant for all statistical analyses. The analyses were performed using the SPSS software package version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of 144 patients with histopathological diagnoses of metastatic squamous cell or undifferentiated carcinomas to cervical lymph nodes without detected primary tumor were included in the study. The mean age was 58.8 years (range, 28-92 years). The majority were males (71.5 %). The most common histology was poorly differentiated squamous cell carcinoma (39.6%). The most common nodal staging was N2b (26.4%), and bilaterality was identified in 17.4% of cases. In addition, 56.9% of the patients had more than 1 cervical lymph node level involved, and the most frequently involved level was II (84%).

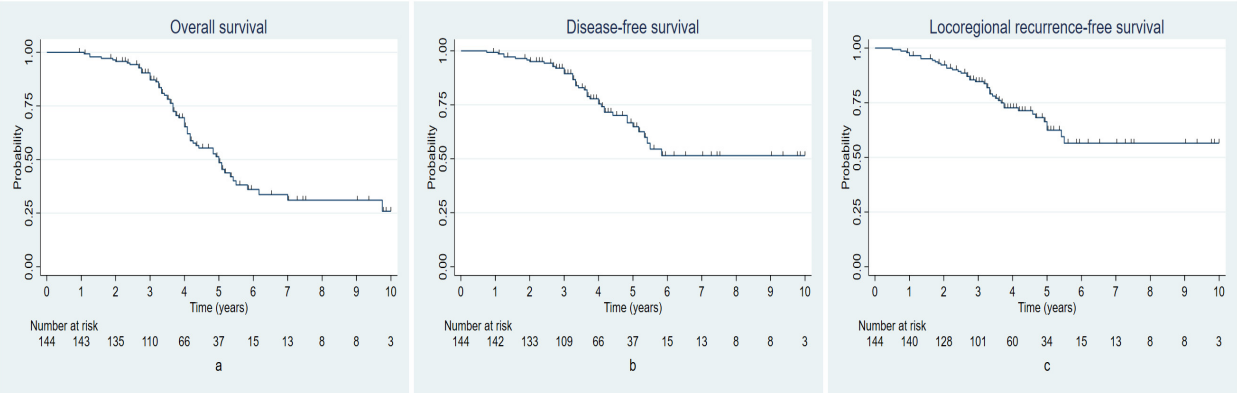

Figure 1. Kaplan-Meier Plot for Overall Survival (a), disease-free survival (b), and locoregional recurrence-free survival (c) of the entire cohort.

Table 1. Demographic, Clinicopathologic, and Treatment Characteristics, Disease Recurrence, and Survival Outcomes of the Entire Cohort

Variables	All patients n=144 (%)
Age (years)	
Mean±SD	58.8±11.9
Range	28-92
Gender	
Male	103 (71.5)
Female	41 (28.5)
Smoking	
Yes	64 (44.4)
No	80 (55.6)
Alcohol	
Yes	53 (36.8)
No	91 (63.2)
Lymph node staging	
N1	18 (12.5)
N2a	22 (15.3)
N2b	38 (26.4)
N2c	22 (15.3)
N3a	8 (5.6)
N3b	36 (25)
Lymph node staging	
N1	18 (12.5)
N2	82 (56.9)
N3	44 (30.6)
ENE	
Yes	36 (25)
No	108 (75)
Laterality of lymph node involvement	
Right	75 (52.1)
Left	44 (30.6)
Bilateral	25 (17.4)
Lymph node level	
I	23 (16)
II	121 (84)
III	69 (47.9)
IV	33 (22.9)
V	31 (21.5)
Largest lymph node diameter (cm)	
Mean±SD	4.34±1.73
Pathology	
SCCA, WD	35 (24.3)
SCCA, MD	19 (13.2)
SCCA, PD	57 (39.6)
UDCA	33 (22.9)
EBER (n=79)	
Positive	10 (12.7)
Negative	69 (87.3)
Treatment	
Surgery alone	2 (1.4)
Radiotherapy alone	18 (12.5)
Surgery+(chemo)radiotherapy	40 (27.8)
Chemoradiotherapy	84 (58.3)
Radiotherapy	
Technique	
2D	39 (27.1)
3D/IMRT	105 (72.9)
Mucosal irradiation	
Total mucosal irradiation	127 (88.2)
Oropharynx-hypopharynx	11 (7.6)
No	6 (4.2)
Disease recurrence	
Primary tumor emergence	6 (4.2)
Neck recurrence	39 (27.1)
Distant metastasis	9 (6.3)
Overall survival	
3-year	87.1
5-year	51.3
Disease-free survival	
3-year	89.4
5-year	64.9
Locoregional recurrence-free survival	
3-year	84.6
5-year	72.7

SCCA, squamous cell carcinoma; WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated; UDCA, undifferentiated carcinoma; EBER, EBV-encoded small RNA; 2D, 2-dimensional radiotherapy; 3D, 3-dimensional conformal radiotherapy; IMRT, Intensity modulated radiotherapy

The most common treatment modality was chemoradiotherapy (58.3%), followed by surgery and (chemo)radiotherapy (27.8%), radiotherapy alone (12.5%), and surgery alone (1.4%). TMI was administered in 88.2% of cases. Eleven patients (7.6%) with only level III and/or IV lymph nodes had radiation limited to the oropharyngeal and hypopharyngeal areas. In addition, 6 patients classified as N1 disease with only level I or II lymph node involvement had no prophylactic mucosal irradiation. The details of patient characteristics are listed in Table 1.

Oncological outcomes

Survival

The median follow-up duration was 45 months (range, 11-147 months). The 3-year and 5-year OS, DFS, and LRRFS of the entire cohort were 87.1% and 51.3%, 89.4% and 64.9%, and 84.6% and 72.7%, respectively (Figure 1).

Primary tumor emergence (PTE)

PTE was detected in 6 patients (4.2%), including

Table 2. Demographic, Clinicopathologic, and Treatment Characteristics, Disease Recurrence, and Survival Outcomes of Patients who had EBER Tests before and after Matching

Variables	Before matching n=79			After matching n=20		
	Negative EBER n=69 (%)	Positive EBER n=10 (%)	p-value	Negative EBER n=10 (%)	Positive EBER n=10 (%)	p-value
Age (years)						
Mean±SD	60.1±12.4	59.6±6.8	0.904	59.4±9.8	59.6±6.8	1.000
Range	31-92	50-70		43-73	50-70	
Gender						
Male	48 (69.6)	8 (80)	0.715	7 (70)	8 (80)	1.000
Female	21 (30.4)	2 (20)		3 (30)	2 (20)	
Smoking						
Yes	36 (52.2)	7 (70)	0.332	6 (60)	7 (70)	1.000
No	33 (47.8)	3 (30)		4 (40)	3 (30)	
Alcohol						
Yes	27 (39.1)	5 (50)	0.515	5 (50)	5 (50)	1.000
No	42 (60.9)	5 (50)		5 (50)	5 (50)	
Lymph node staging						
N1	7 (10.1)	0	0.024	0	0	0.378
N2a	10 (14.5)	0		2 (20)	0	
N2b	15 (21.7)	0		0	0	
N2c	12 (17.4)	1 (10)		0	1 (10)	
N3a	4 (5.8)	3 (30)		3 (30)	3 (30)	
N3b	21 (30.4)	6 (60)		5 (50)	6 (60)	
Lymph node staging						
N1	7 (10.1)	0	0.006	0	0	1.000
N2	37 (53.6)	1 (10)		2 (20)	1 (10)	
N3	25 (36.2)	9 (90)		8 (80)	9 (90)	
ENE						
Yes	21 (30.4)	6 (60)	0.082	5 (50)	6 (60)	1.000
No	48 (69.6)	4 (40)		5 (50)	4 (40)	
Laterality of lymph node involvement						
Right	31 (44.9)	6 (60)	0.616	5 (50)	6 (60)	0.745
Left	24 (34.8)	3 (30)		4 (40)	3 (30)	
Bilateral	14 (20.3)	1 (10)		1 (10)	1 (10)	
Lymph node level						
I	15 (21.7)	1 (10)	0.677	4 (40)	1 (10)	0.303
II	57 (82.6)	10 (100)	0.345	10 (100)	10 (100)	1.000
III	44 (63.8)	8 (80)	0.480	9 (90)	8 (80)	1.000
IV	23 (33.3)	4 (40)	0.728	8 (80)	4 (40)	0.170
V	23 (33.3)	6 (60)	0.159	6 (60)	6 (60)	1.000
Largest lymph node diameter (cm)						
Mean±SD	4.5±1.7	6.6±1.6	<0.001	5.6±1.2	6.6±1.6	0.168
Pathology						
SCCA, WD	11 (15.9)	0	0.210	1 (10)	0	0.787
SCCA, MD	5 (7.2)	1 (10)		0	1 (10)	
SCCA, PD	28 (40.6)	4 (40)		2 (20)	4 (40)	
UDCA	25 (36.2)	5 (50)		7 (70)	5 (50)	

Table 2. Continued

Variables	Before matching n=79			After matching n=20		
	Negative EBER n=69 (%)	Positive EBER n=10 (%)	p-value	Negative EBER n=10 (%)	Positive EBER n=10 (%)	p-value
Treatment						
Radiotherapy alone	7 (10.1)	0	0.147	0	0	0.100
Surgery alone	0	0		0	0	
Surgery+(chemo)radiotherapy	16 (23.2)	2 (20)		3 (30)	2 (20)	
Chemoradiotherapy	46 (66.7)	8 (80)		7 (70)	8 (80)	
Radiotherapy						
Technique						
2D	12 (17.4)	0	0.345	1 (10)	0	1.000
3D/IMRT	57 (82.6)	10 (100)		9 (90)	10 (100)	
Mucosal irradiation						
Total mucosal irradiation	61 (88.4)	10 (100)	0.343	10 (100)	10 (100)	1.000
Oropharynx-hypopharynx	7 (10.1)	0		0	0	
No	1 (1.4)	0		0	0	
Treatment toxicity						
Acute toxicity						
Grade <3	66 (95.7)	10 (100)	0.987	10 (100)	10 (100)	1.000
Grade 4	3 (4.3)	0		0	0	
Late toxicity						
Grade <3	68 (98.6)	10 (100)	1.000	10 (100)	10 (100)	1.000
Grade 4	1 (1.4)	0		0	0	
Disease recurrence						
Primary tumor emergence	3 (4.3)	0	1.000	0	0	-
Neck recurrence	25 (36.2)	4 (40)	1.000	6 (60)	4 (40)	0.656
Distant metastasis	5 (7.2)	1 (10)	0.569	1 (10)	1 (10)	1.000
Overall survival						
3-year	86.5	90	0.208	80	90	0.196
5-year	36.4	57.1		30	57.1	
Disease-free survival						
3-year	89.5	90	0.537	80	90	0.176
5-year	47.2	66.7		35	66.7	
Locoregional recurrence-free survival						
3-year	81.9	90	0.506	80	90	0.264
5-year	50.8	37.5		45	37.5	

SCCA, squamous cell carcinoma; WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated; UDCA, undifferentiated carcinoma; EBER, EBV-encoded small RNA; 2D, 2-dimensional radiotherapy; 3D, 3-dimensional conformal radiotherapy; IMRT, Intensity modulated radiotherapy

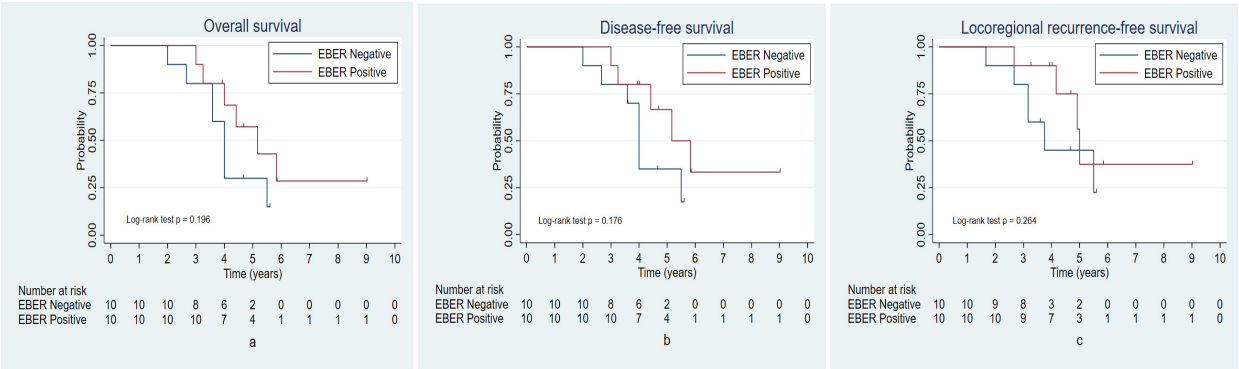

Figure 2. Kaplan-Meier Plot for Overall Survival (a), disease-free survival (b), and locoregional recurrence-free survival (c) according to the EBER status of patients after matched pair analysis.

Table 3. Comparison of Demographic, Clinicopathologic, and Treatment Characteristics, Disease Recurrence, and Survival Outcomes between Studies from Non-Endemic NPC, Endemic-NPC Countries, and the Present Study

Variables	Non-endemic NPC countries Average (range) (%)	Endemic NPC countries Average (range) (%)	The present study (%)
Mean age (years)	61.5 (56-69)	55.4 (55-57)	58.8
Gender			
Male	80.6 (72-89)	76.9 (70-82)	71.5
Female	19.4 (11-28)	23.1 (18-30)	28.5
Nodal stage			
N1	15.9 (12-19)	21.3 (6-51)	12.5
N2	56.2 (43-75)	67.3 (65-85)	56.9
N3	26.9 (13-43)	11.4 (6-29)	30.6
Lymph node level			
I	6.3 (4-11)	9.3 (6-11)	16
II	71.1 (46-88)	55.8 (33-95)	84
III	43.2 (28-62)	35.3 (34-42)	47.9
IV	13.6 (9-15)	16.6 (13-24)	22.9
V	10.7 (6-13)	3.2 (2-11)	21.5
Histopathological diagnosis			
SCCA WD	18.8 (12-49)	19.7 (8-67)	24.3
SCCA MD	30.7 (16-82)	24.1 (23-30)	13.2
SCCA PD	42.6 (39-44)	45.7 (10-65)	39.6
UD	7.9 (6-9)	10.5 (10-11)	22.9
Treatment			
Surgery alone	8.5 (4-10)	19.4 (7-35)	1.4
Radiotherapy alone	14.9 (1-36)	23.1 (10-78)	12.5
Surgery+(chemo)radiotherapy	49.2 (29-58)	41.6 (14-67)	27.8
Chemoradiotherapy	27.4 (8-56)	15.9 (6-100)	58
Disease recurrence			
Primary tumor emergence	8.2 (1-12)	10.5 (4-22)	4.2
Neck recurrence	15.2 (9-25)	21.9 (10-42)	27.1
Distant metastasis	11.5 (1-26)	9.9 (4-28)	6.3
5-year overall survival	52.5 (30-85)	60.7 (40-86)	51.3
5-year disease-free survival	60.2 (49-77)	66.4 (29-84)	64.9

Studies from non-endemic NPC countries (Koivunen et al., 2002; Boscolo-Rizzo et al., 2006; Axelsson et al., 2017; Al Kadah et al ., 2017; Mizuta et al., 2018; Dorobisz et al., 2019; Ryan et al., 2019; Lee et al., 2020; Sprave et al., 2020); Studies from endemic countries (Huang et al., 2008; Lu H et al., 2009; Lu X et al., 2009; Lou et al., 2015; Hung et al., 2018; Wang et al., 2018; Dou et al., 2020; Li et al., 2022); NPC, nasopharyngeal cancer; SCCA, squamous cell carcinoma; WD, well-differentiated; MD; moderately differentiated; PD, poorly differentiated; UDCA, undifferentiated carcinoma

the floor of the mouth in 2 patients and the oral tongue, nasopharynx, supraglottis, and pyriform sinus, in 1 patient at each site. The average time of PTE was 21.3 months (range, 12-42 months). Notably, these 6 patients did not have TMI.

Neck persistence/recurrence

Cervical lymph node persistence or recurrence was recorded in 39 patients (27.1%) with an average time of 19.6 months (range, 6-56 months).

Distant metastasis

Nine patients (6.3%) developed distant organ metastasis, including lung (4 patients), mediastinal lymph node (2 patients), inguinal lymph node (1 patient), axillary lymph node (1 patient), liver (1 patient), and adrenal gland (1 patient). The average time of distant metastasis was 25.1 months (range, 12-50 months). Notably, 4 patients with PTE also developed distant organ metastasis.

Independent prognostic factors for survival outcomes

Multivariate Cox proportional hazard regression model analysis was performed based on clinicopathological variables for the different survival criteria. The analysis revealed that extranodal extension (ENE) and N3 compared to N1 were independent prognostic factors for OS (HR 2.90, 95% CI 1.12-7.51, p = 0.028 and HR 3.66, 95%CI 1.23-11.89, p = 0.031, respectively). ENE was the only factor affecting DFS (HR 3.05, 95%CI 1.05-8.81, p = 0.040). However, ENE had only a trend of affecting

LRRFS (HR 2.91, 95%CI 0.99-8.46, p = 0.050).

Propensity-matched pair analysis of patients who had EBER test

Histochemical staining for EBER was carried out in seventy-nine patients (54.9%), and the result was positive in 10 patients (12.7%). There were differences in clinicopathological characteristics of the patients with positive and negative EBER staining, as presented in Table 2. The propensity score matching analysis was performed, which involved two steps. In the first step, the likelihood that a patient would have an EBER-positive result was assessed using a logistic regression model as a function of age, gender, history of smoking and alcohol usage, lymph node staging, level of lymph node involvement, largest lymph node diameter, pathology, treatment modality, and follow-up time. From this regression, the predicted probability of having an EBER-positive result, or propensity score, was calculated for each patient. In the second step, patients were matched 1:1 from both groups based on the propensity scores with a caliper width of 0.25 SD. In the case of more than two matches, one pair was picked randomly from among all potential matches. Cases without a matched control were excluded.

The final matched pair analysis included 20 patients (10 patients in each group), and patient characteristics before and after matching are listed in Table 2.

Oncological outcomes

Survival

The median follow-up duration was 48 months (range, 24-108 months). The 3-year and 5-year OS, DFS, and LRRFS of EBER-negative patients were 80% and 30%, 80% and 35%, and 80% and 45%, respectively, while those of EBER-positive patients were 90% and 57.1%, 90% and 66.7%, and 90% and 37.5%, respectively. Notably, there were no statistically significant differences between the two groups in all survival outcomes (Figure 2).

Primary tumor emergence

No PTE was detected in any patient. Notably, all 20 patients received prophylactic TMI.

Neck persistence/recurrence

Cervical lymph node persistence or recurrence was detected more frequently in the EBER-negative group than in the EBER-positive group (60% and 40%, respectively) but without statistical significance (p = 0.656).

Distant metastasis

Distant metastasis was detected in 2 patients (1 in each group) in the lung and inguinal lymph nodes.

Independent prognostic factors for survival outcomes

The Cox proportional hazard regression model analysis was performed based on the EBER status, clinicopathological variables, and treatment modality for the OS, DFS, and LRRFS. The univariate and multivariate analyses revealed that no factor significantly influenced any survival outcomes.

Discussion

This multicenter study, conducted in an area of endemic nasopharyngeal carcinoma (NPC), evaluated the clinicopathological characteristics, oncological outcomes, and prognostic predictors of HNCUP. A literature review of HNCUP series published from non-endemic NPC countries and endemic NPC countries was performed and compared to the present cohort in Table 3. The results revealed that most of the characteristics were in line with ours, the exception being that we had more patients with level I and V lymph nodes and undifferentiated carcinoma. However, the discordant results may be because some studies included other histopathological diagnoses such as adenocarcinoma, malignant melanoma, mucoepidermoid carcinoma, and neuroendocrine carcinoma [10, 22, 23]. In addition, only 2 studies from non-endemic NPC countries [10, 24] and 1 study from endemic NPC countries [25] included undifferentiated carcinoma in the analysis.

The OS and DFS in the present study were in the same range as studies from both endemic and non-endemic NPC countries (Table 3). ENE and N stage were the independent prognostic predictors for OS and DFS of the present study, findings concordant with studies from other endemic NPC countries [26-29, 22, 30]. In contrast, p16 status and N stage were the common predictors of survival in studies from non-endemic NPC countries [6, 31, 16, 11].

PTE was detected in 6 patients (4.2%) in the present study. The most common site was the oral cavity (3 patients); others were the nasopharynx, supraglottis, and pyriform sinus (1 patient at each location). One patient with nasopharyngeal tumor emergence was treated with limited mucosal irradiation. Studies from endemic NPC countries reported a rate of PTE of 4-22% [26, 27, 29, 25]. Nasopharyngeal carcinoma was the most common tumor (20-50%), followed by hypopharyngeal/laryngeal carcinoma (31%), oral cavity carcinoma (30%), and oropharyngeal carcinoma (10-24%) [26, 27, 29, 25]. In contrast, studies from non-endemic NPC countries reported a lower rate of PTE of 1-12%, and the most common site was the oropharynx, followed by the hypopharynx and oral cavity [6, 10, 31, 32].

Our results revealed a neck recurrence rate of 27.1% which was in the same range as studies from endemic NPC countries reporting a rate of 10-42% [27, 29, 25, 18, 24]. In contrast, studies from non-endemic NPC countries reported a lower rate of 9-25% [10, 31, 32].

We detected distant metastatic disease after treatment in 9 patients (6.3%), the most common site being the lung (4 patients), followed by the mediastinal lymph nodes (2 patients). The distant metastasis rate reported from endemic NPC countries was 4-28%, and the most common organ was the lung, followed by the liver, bone, and mediastinal lymph node [29, 25, 22]. In addition, studies from non-endemic NPC countries reported a similar rate of 1-26% with similar organs, including lung, bone, and liver [3, 10, 31, 32].

In recent years, Human papillomavirus (HPV) has been identified as a common cause of oropharyngeal cancer; therefore, there has been growing interest in the importance of HPV in association with HNCUP,

particularly in Western countries [31]. In studies from non-endemic NPC countries, prophylactic mucosal irradiation usually only included the oropharynx and hypopharynx. At the same time, the nasopharynx would be irradiated when level V or retropharyngeal lymph nodes were detected [6, 10, 31, 32]. As a result, the most common PTE in these studies was the oropharynx, followed by the hypopharynx and oral cavity [6, 10, 31, 30]. In contrast, most studies from endemic NPC countries routinely included the nasopharynx in the radiation field [26-28, 25, 18, 30]. Although the nasopharynx was still the most common site of tumor emergence, the majority of cases were detected in patients without nasopharyngeal mucosa irradiation [27, 29, 25]. In addition, the analysis revealed that TMI, which included nasopharyngeal mucosa, improved overall survival and locoregional control [27, 29, 30].

PTE was detected in 6 patients (4.2%) out of the whole cohort in this study which was a relatively low incidence compared with previous studies (Table 3), and all these patients did not receive TMI. The matched pair analysis showed no significant differences in oncological outcomes between the EBER-positive and EBER-negative groups and no independent prognostic factors for survival outcomes. In addition, no PTE was observed in either group. However, all patients in both groups received TMI.

The present study has some limitations. First, it is a retrospective study with relative-ly few patients, particularly those included in the matched pair analysis. Second, a few pa-tients had FDG PET/CT and HPV tests performed. Third, treatment selection was based on the preference of the physicians at each center. Finally, most patients (88.2%) in the study received TMI. Therefore, to explore the influence of radiation volume on PTE between EBER-positive and -negative patients, a multicenter, prospective, randomized, controlled trial is warranted.

The survival outcomes of studies from endemic and non-endemic NPC countries and the present study were along the same lines. We observed a relatively low rate of primary tumor emergence, which occurred only in patients who did not have prophylactic total mucosal irradiation. The matched pair analysis of EBER-positive and -negative groups revealed comparable oncological outcomes and no primary tumor emergence when total mucosal irradiation was administered. Therefore, total mucosal irradiation may be considered in HNCUP patients who live in endemic NPC countries independent of EBER status.

Author Contribution Statement

P.S.: Conceptualization, Methodology, formal analysis, Writing- Original draft preparation, Supervision, D.R.: Conceptualization, Investigation, Writing - review& editing, I.C.: Investigation, Methodology, P.M.: Investigation, Data curation, K.K.: Investigation, Data curation, R.B.: Investigation, Data curation, C.S.: Investigation, Data curation, Formal analysis. S.D.: Investigation, Data curation, T.A.: Investigation, Data curation, All authors reviewed and approved the final manuscript.

Acknowledgements

General

The authors would like to thank Mr. Phatsaworn Sangthongluan, B.Sc. (Biostatistics), for his assistance in data analysis.

Competing interests

The authors of this study have no conflicts of interest to declare.

Ethical approval

The Ethics Committee of the Faculty of Medicine, Chiang Mai University, and all the participating centers approved the study protocol. The reference number is 398/2021. All procedures involving human participants were in accordance with the ethical standards of the National Research Committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent

The informed consent has been waived by the ethics board of the institute considering this as a retrospective study.

Availability of data and materials

All data will be made available by the corresponding author upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Pavlidis N, Pentheroudakis G. Cancer of unknown primary site. Lancet. 2012;379(9824):1428-35. https://doi.org/10.1016/s0140-6736(11)61178-1.
2. Grau C, Johansen LV, Jakobsen J, Geertsen P, Andersen E, Jensen BB. Cervical lymph node metastases from unknown primary tumours. Results from a national survey by the danish society for head and neck oncology. Radiother Oncol. 2000;55(2):121-9. https://doi.org/10.1016/s0167-8140(00)00172-9.
3. Rödel RM, Matthias C, Blomeyer BD, Wolff HA, Jung K, Christiansen H. Impact of distant metastasis in patients with cervical lymph node metastases from cancer of an unknown primary site. Ann Otol Rhinol Laryngol. 2009;118(9):662-9. https://doi.org/10.1177/000348940911800911.
4. Haas I, Hoffmann TK, Engers R, Ganzer U. Diagnostic strategies in cervical carcinoma of an unknown primary (cup). Eur Arch Otorhinolaryngol. 2002;259(6):325-33. https://doi.org/10.1007/s00405-002-0470-1.
5. Koivunen P, Laranne J, Virtaniemi J, Bäck L, Mäkitie A, Pulkkinen J, et al. Cervical metastasis of unknown origin: A series of 72 patients. Acta Otolaryngol. 2002;122(5):569-74. https://doi.org/10.1080/00016480260092435.
6. Boscolo-Rizzo P, Da Mosto MC, Gava A, Marchiori C. Cervical lymph node metastases from occult squamous cell carcinoma: Analysis of 82 cases. ORL J Otorhinolaryngol Relat Spec. 2006;68(4):189-94. https://doi.org/10.1159/000091394.
7. Robbins KT, Clayman G, Levine PA, Medina J, Sessions R, Shaha A, et al. Neck dissection classification update: Revisions proposed by the american head and neck society and the american academy of otolaryngology-head and neck

surgery. Arch Otolaryngol Head Neck Surg. 2002;128(7):751-8. https://doi.org/10.1001/archotol.128.7.751.
8. Doty JM, Gossman D, Kudrimoti M, Valentino J, Arnold S, Spring PM. Analysis of unknown primary carcinomas metastatic to the neck: Diagnosis, treatment, and outcomes. J Ky Med Assoc. 2006;104(2):57-64.
9. Jereczek-Fossa BA, Jassem J, Orecchia R. Cervical lymph node metastases of squamous cell carcinoma from an unknown primary. Cancer Treat Rev. 2004;30(2):153-64. https://doi.org/10.1016/j.ctrv.2003.10.001.
10. Al Kadah B, Papaspyrou G, Linxweiler M, Schick B, Rübe C, Büchler BS, et al. Cancer of unknown primary (cup) of the head and neck: Retrospective analysis of 81 patients. Eur Arch Otorhinolaryngol. 2017;274(6):2557-66. https://doi.org/10.1007/s00405-017-4525-8.
11. Lee MY, Fowler N, Adelstein D, Koyfman S, Prendes B, Burkey BB. Detection and oncologic outcomes of head and neck squamous cell carcinoma of unknown primary origin. Anticancer Res. 2020;40(8):4207-14. https://doi.org/10.21873/anticanres.14421.
12. Christiansen H, Hermann RM, Martin A, Nitsche M, Schmidberger H, Pradier O. Neck lymph node metastases from an unknown primary tumor retrospective study and review of literature. Strahlenther Onkol. 2005;181(6):355-62. https://doi.org/10.1007/s00066-005-1338-2.
13. Kwee TC, Kwee RM. Combined fdg-pet/ct for the detection of unknown primary tumors: Systematic review and meta-analysis. Eur Radiol. 2009;19(3):731-44. https://doi.org/10.1007/s00330-008-1194-4.
14. Rudmik L, Lau HY, Matthews TW, Bosch JD, Kloiber R, Molnar CP, et al. Clinical utility of pet/ct in the evaluation of head and neck squamous cell carcinoma with an unknown primary: A prospective clinical trial. Head Neck. 2011;33(7):935-40. https://doi.org/10.1002/hed.21566.
15. Mehta V, Johnson P, Tassler A, Kim S, Ferris RL, Nance M, et al. A new paradigm for the diagnosis and management of unknown primary tumors of the head and neck: A role for transoral robotic surgery. Laryngoscope. 2013;123(1):146-51. https://doi.org/10.1002/lary.23562.
16. Ryan JF, Motz KM, Rooper LM, Mydlarz WK, Quon H, Gourin CG, et al. The impact of a stepwise approach to primary tumor detection in squamous cell carcinoma of the neck with unknown primary. Laryngoscope. 2019;129(7):1610-6. https://doi.org/10.1002/lary.27625.
17. Chernock RD, Lewis JS. Approach to metastatic carcinoma of unknown primary in the head and neck: Squamous cell carcinoma and beyond. Head Neck Pathol. 2015;9(1):6-15. https://doi.org/10.1007/s12105-015-0616-2.
18. Dou S, Li R, Zhang L, Wang Z, Xie L, Zhang C, et al. Long-term results of elective mucosal irradiation for head and neck cancer of unknown primary in chinese population: The emicup study. Cancer Med. 2020;9(5):1712-20. https://doi.org/10.1002/cam4.2856.
19. Mahdavifar N, Ghoncheh M, Mohammadian-Hafshejani A, Khosravi B, Salehiniya H. Epidemiology and inequality in the incidence and mortality of nasopharynx cancer in asia. Osong Public Health Res Perspect. 2016;7(6):360-72. https://doi.org/10.1016/j.phrp.2016.11.002.
20. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492.
21. Patel SG LW, Ridge JA, et al. Cervical lymph nodes and unknown primary tumors of the head and neck. In ajcc cancer staging manual, eds amin mb, edge s, greene f, et al. 8th ed. New York: Springer; 2017. pp 69-78
22. Wang Y, He SS, Bao Y, Cai XY, Chen HY, Yang XL, et al. Cervical lymph node carcinoma metastasis from unknown primary site: A retrospective analysis of 154 patients. Cancer Med. 2018;7(5):1852-9. https://doi.org/10.1002/cam4.1458.
23. Dorobisz K, Wlodarska-Polinska I, Pazdro-Zastawny K, Rutkowski T, Palka P, Dworzecki T, et al. The impact of the patient's condition, diagnostic procedures and treatment on the survival of carcinoma of unknown primary site patients. Cancer Manag Res. 2019;11:6603-14. https://doi.org/10.2147/cmar.S204346.
24. Sprave T, Rühle A, Hees K, Kalckreuth T, Verma V, Stoian R, et al. Radiotherapeutic management of cervical lymph node metastases from an unknown primary site – experiences from a large cohort treated with modern radiation techniques. Radiat Oncol. 2020;15(1):80. https://doi.org/10.1186/s13014-020-01529-z.
25. Hung YH, Liu SA, Wang CC, Wang CP, Jiang RS, Wu SH. Treatment outcomes of unknown primary squamous cell carcinoma of the head and neck. PLoS One. 2018;13(10):e0205365. https://doi.org/10.1371/journal.pone.0205365.
26. Huang CC, Tseng FY, Yeh TH, Wen YH, Hsu CJ, Ko JY, et al. Prognostic factors of unknown primary head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2008;139(3):429-35. https://doi.org/10.1016/j.otohns.2008.05.015.
27. Lu H, Yao M, Tan H. Unknown primary head and neck cancer treated with intensity-modulated radiation therapy: To what extent the volume should be irradiated. Oral Oncol. 2009;45(6):474-9. https://doi.org/10.1016/j.oraloncology.2008.06.006.
28. Lu X, Hu C, Ji Q, Shen C, Feng Y. Squamous cell carcinoma metastatic to cervical lymph nodes from an unknown primary site: The impact of radiotherapy. Tumori. 2009;95(2):185-90. https://doi.org/10.1177/030089160909500209.
29. Lou J, Wang S, Wang K, Chen C, Zhao J, Guo L. Squamous cell carcinoma of cervical lymph nodes from an unknown primary site: The impact of neck dissection. J Cancer Res Ther. 2015;11 Suppl 2:C161-7. https://doi.org/10.4103/0973-1482.168178.
30. Li R, Liao K, Wei Z, Liu Z, He Y, Wang J, et al. The prognostic role of radiotherapy and radiotherapy target in cervical lymph node metastatic squamous cell carcinoma with unknown primary: A retrospective study. J Cancer Res Clin Oncol. 2022;148(6):1437-45. https://doi.org/10.1007/s00432-021-03724-1.
31. Axelsson L, Nyman J, Haugen-Cange H, Bove M, Johansson L, De Lara S, et al. Prognostic factors for head and neck cancer of unknown primary including the impact of human papilloma virus infection. J Otolaryngol Head Neck Surg. 2017;46(1):45. https://doi.org/10.1186/s40463-017-0223-1.
32. Mizuta M, Kitamura M, Tateya I, Tamaki H, Tanaka S, Asato R, et al. Unknown primary squamous cell carcinoma of the head and neck: Retrospective analysis of 80 cases. Acta Otolaryngol. 2018;138(6):590-6. https://doi.org/10.1080/00016489.2017.1422141.