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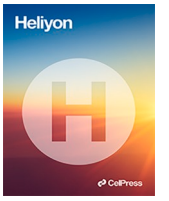

Research article

Distribution of Epstein-Barr virus in the oral cavity of Thais with various oral mucosal conditions

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ABSTRACT

Objectives: We aimed to examine the presence of EBV, EBV strains, and variants among 3 oral conditions including normal oral mucosa (NOM), oral potentially malignant disorders/oral cancer (OPMDs/OC) and non-OPMDs/OC in a group of Thais.

Material and methods: Oral exfoliated cells were obtained from 315 participants living in the northeastern and central regions of Thailand. The participants were divided into 3 groups encompassing the NOM, the OPMDs/OC and the non-OPMDs/OC groups. The presence of EBV was first determined by PCR using primers for *LMP1* gene. Subsequently, EBV strains of *EBNA3c* and variants based on *LMP1* sequences were determined by real-time PCR.

Results: The prevalence of EBV in OPMDs/OC, non-OPMDs/OC and NOM were 72.0 %, 56.2 %, and 27.2 % respectively. EBV type A, B and AB were found in 52.1 %, 32.1 % and 15.8 % of all positive samples, respectively. The percentage of participants with EBV type A was more prominent in the NOM group (72.0 %) compared to the non-OPMDs/OC (54.8 %) and the OPMDs/OC group (41.8 %) whereas EBV type B was higher in the OPMDs/OC group (35.8 %) compared to the non-OPMDs/OC (31.5 %) and the NOM (24.0 %) groups. Regarding EBV variants, 30-bp deletion *LMP1* variant (del-LMP1) which is more associated with malignant transformation was predominately found in the OPMDs/OC (32.8 %) and the non-OPMDs/OC (38.4 %) groups compared to the NOM group (20.0 %).

Conclusions: High frequency of EBV was demonstrated in the OPMDs/OC group. EBV type A was more predominant in the NOM group whereas EBV type B was more prevalent in the OPMDs/OC group. The del-LMP1 variant was more common in the OPMDs/OC and the non-OPMDs/OC groups.

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1. Introduction

In 2021, oral cancer (OC) was the 16th most commonly diagnosed cancer worldwide, with 377,713 new cases per year [1]. Oral squamous cell carcinoma (OSCC) is the most common OC, originating from squamous epithelial cells. Frequently, OSCC is preceded by oral potentially malignant disorders (OPMDs). The incidence of OC is high among Asia-Pacific countries, particularly in South and Southeast Asia [2]. In Thailand, OC is also one of the most common malignancies, especially in the northeastern region [3]. Tobacco smoking, betel quid chewing, and alcohol drinking are known main risk factors for both OC and OPMDs [4]. Epstein-Barr virus (EBV) has also been suggested as one of the etiologic factors of oral malignancy. However, previous studies exploring the association between EBV and OC showed controversial findings [5,6].

EBV, also known as human gammaherpesvirus 4, was firstly isolated from African Burkitt's lymphoma. Following initial infection, EBV maintains a lifelong latent phase in which it targets epithelial cells and B lymphocytes [7]. Different EBV strains has been reported. EBV type A and B, often known as type 1 and type 2, respectively, are EBV subtypes characterized by polymorphisms in the *Epstein-Barr nuclear antigen* (*EBNA*) gene. EBV type A is prevalent in Western and Asian countries and can increase the ability to transform B cells. EBV type B, on the other hand, is more frequent in Africa and has a lower ability to transform the host's cell [8]. Recent EBV research has focused on polymorphisms in the *latent membrane protein 1* (*LMP1*). A 30-bp deletion in the *LMP1* gene has been linked to several epithelial cancers, including nasopharyngeal carcinoma (NPC) and gastric carcinoma [9,10]. This variant has a greater oncogenic ability than the regular *LMP1*.

Currently, there is no information on the prevalence of EBV, EBV strains and variants among OPMDs and OC in the Thai population. Therefore, we investigated the prevalence of EBV, as well as the distribution of strains and variants among OPMDs/OC, non-OPMDs/OC compared to the normal oral mucosa (NOM). Furthermore, factors associated with the presence of EBV was investigated.

2. Materials and methods

2.1. Study population

This study was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COA. No.MU-DT/PY-IRB 2019/050.3107 and COA.No.MU-DT/PY-IRB 2019/041.0307). Each subject acknowledged the protocol verbally and in writing and then written informed consent forms were signed. The ethical guidelines of the Declaration of Helsinki were followed throughout the study.

Study participants include those who attended OPMDs/OC screening in the "Development of Disease Management Model for Oral Cancer with an Integration Network of Screening, Surveillance, and Treatment from Primary Care Unit to Tertiary Care in North East Health District" project (n = 260) [11] and the participants at the Faculty of Dentistry, Mahidol University (n = 55). Exclusion criteria included participants with a history or clinical evidence of EBV-associated infection and a history of immunosuppression.

All participants were divided into three groups as follows.

1) The OPMDs/OC group (n = 93): Participants in this group had either OPMDs (leukoplakia (n = 35), erythroplakia (n = 9), proliferative verrucous leukoplakia (n = 7), oral lichen planus (n = 5), oral lichenoid lesions (n = 15), oral submucous fibrosis (n = 2), lupus erythematosus (n = 2), chronic graft-versus-host disease (n = 1), and actinic cheilitis (n = 3) or OC (n = 14).
2) Non-OPMDs/OC (n = 130): This group included participants with any oral lesions being neither OPMDs nor OC. These included oral candidiasis (n = 40), irritation fibroma (n = 33), squamous papilloma (n = 13), pyogenic granuloma (n = 10), intramucosal nevus (n = 1), frictional keratosis (n = 20), traumatic ulcer (n = 12), and mucocele (n = 1).
3) Normal oral mucosa (NOM) (n = 92): This group included individuals with normal oral mucosa with or without risk factor for OC.

A history of OC risk factors such as tobacco smoking, use of smokeless tobacco, alcohol consumption, betel quid chewing, and prolong working in the field with sunlight more than four days per week, was collected.

2.2. Sample collection and DNA extraction

The buccal collection brush (QIAgen®, Maryland, USA) was used to collect the oral mucosal cells according to manufacturer's instructions. Subsequently, the collection brush was cut short with sterile scissors and put into a 1.5 mL sterile tube for further processing. The obtained samples were stored at −20 °C until DNA extraction was performed.

DNA was extracted and purified using a commercial Gentra Puregene Buccal kit (QIAgen®, Maryland, USA) according to the manufacturer's protocol. The nanodrop (NanoDrop 2000c Spectrophotometer, Thermo Scientific™, USA) was used to quantify the genomic DNA collected. The DNA was kept at −20 °C until further investigation.

2.3. Presence of EBV by polymerase chain reaction (PCR)

PCR with *LMP1* primers was conducted for the presence of EBV in the oral epithelial cells. *GAPDH* primers were used to determine DNA integrity (Table 1). PCR mixtures contained 10x PCR buffer, 0.2 μL of 10 mM dNTPs, 0.1 μL of 25 mM primers, 0.5 unit of Taq DNA polymerase (Thermo Scientific™, Vilnius, Lithuania), 100 ng of DNA template, and water to a final volume of 10 μL. The primer sequences, PCR product length and thermocycle protocol were used as described previously with some modifications (Table 1) [12].

DNA obtained from a nasopharyngeal carcinoma and deionized water were utilized as positive and negative controls, respectively. Gel electrophoresis on a 2 % agarose gel was used to evaluate the PCR results and visualized under UV transillumination (Amersham ™ ImageQuant™ 800 Flour, Cytiva, USA).

2.4. EBV strain and variant analysis by real-time PCR

Real-time PCR (qPCR) was utilized in EBV positive samples to identify EBV type A and B using primers targeting the *EBNA3C* region (Table 1). *LMP1*_v primers were used for the identification of EBV variants including wild type *LMP1* (wt-LMP1) and its mutant (30-bp deletion at *LMP1* or del-LMP1) as previously described [10]. Briefly, the reaction mixture for the qPCR comprised 10 μL of each master mix containing 5 μL of Luna® Universal qPCR Master Mix (New England Biolabs, MA, USA), 100 ng of DNA, and 0.5 μL of 5 μM of each primer. The qPCR was completed using the CFX96™ Real-Time system (BIO-RAD Hercules, CA, USA). Thermocycling conditions are shown in Table 1. A negative control without DNA sample and a standard EBV type A and wild-*LMP1* controls from a nasopharyngeal specimen were included for every qPCR experiment. The melting curve was analyzed using CFX Manager™ Software (Bio-Rad). The findings were confirmed with gel electrophoresis as previously mentioned.

2.5. PCR reliability and DNA sequencing

All experiments were performed in duplicate. To confirm that the amplicons harbored *LMP1*, *EBNA-3C*, and *LMP1* 30-bp deletion, the positive control samples and the PCR products were randomly selected for sequencing by Macrogen, Seoul, Korea. The DNA sequence findings were compared to other EBV strains and variants to discover the difference in nucleotide sequences.

2.6. Statistical analysis

Statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, US). Descriptive statistical analyses are presented as frequencies, percentages, and median. The normality of all continuous data was analyzed using the Shapiro-Wilk. Comparison of ages was performed using Mann-Whitney *U* test. Differences in categorical variables between groups were assessed using chi-square or Fisher's exact test as appropriate. Association of the presence of EBV and risk factors for OC was explored using binary logistic regression. A p-value of <0.05 was considered statistically significant for all analyses.

Table 1
Primer sequences and PCR conditions.

Primer	Sequence	Product size (base pairs)	Target region	Thermocycler protocol
Presence of EBV analysis by PCR				
***LMP 1*-F**	CCAGACAGCAGCCAACAATTGTC	129	*LMP 1*	95 °C (15 min) 45 cycles of [95 °C (30 s) 56 °C (30 s) 72 °C (30 s)] 72 °C (7 min)
***LMP 1*-R**	GGTAGAAGACCCCCTCTTAC			
***GAPDH*-F**	TGAGGCTCCCACCTTTCTCATC	150	*GAPDH*	95 °C (15 min) 45 cycles of [95 °C (45 s) 56 °C (45 s) 72 °C (45 s)] 72 °C (7 min)
***GAPDH*-R**	TGAGGCCCTGCAGCGTACTC			
EBV strain and variant analyses by qPCR				
***EBNA3C*–F**	GAGAAGGGGAGCGTGTGTTGT		EBNA3C	95 °C (3 min)
		153	Type A	40 cycles of
		246	Type B	[95 °C (10 s) 58 °C (30 s) 95 °C (30 s)] 65 °C (30 s)
***EBNA3C*–R**	GGTAGAAGACCCCCTCTTAC			
***LMP1*_v-F**	GAAGAGGTTGAAAACAAAGGA	186	wt-LMP1	95 °C (3 min)
		156	del-LMP1	40 cycles of [95 °C (10 s) 54 °C (30 s) 95 °C (30 s)] 65 °C (30 s)
***LMP1*_v-R**	GTCATAGTAGCTTAGCTGAAC			

3. Results

3.1. Participant characteristics

The genomic DNA from oral epithelial specimens of all 315 individuals were positive for *GAPDH* amplification. A representative picture illustrating PCR product of *GAPDH* is shown in Fig. 1A (upper panel). Table 2 shows demographic data and risk factors of OC of all participants. The majority of participants were female (72.7 %), with median age of 64 years old. Approximately 50.3 % of participants reported long exposure to sunlight during the day. Betel quid chewing habit and alcohol drinking were present in 32.7 % and 23.2 % of all participants, respectively. There were missing data in 1 participant in some categories, therefore, in some variables, the number was 314 instead of 315 (Table 2).

3.2. Prevalence of EBV in the oral cavity

Using PCR analysis, EBV DNA was detected in 165 of 315 (52.4 %) samples. As depicted in Fig. 1A (lower panel) and Table 2, the prevalence of EBV in the oral cavity of the OPMDs/OC and the non-OPMDs/OC groups were 72.0 % and 56.2 %, respectively, whereas 27.2 % of participants in the NOM group were positive for EBV (Fig. 1B). The prevalence of EBV among different oral mucosal conditions was significantly different ($p < 0.001$).

3.3. Identification of EBV strains and variants

EBV strains and variants were verified in EBV positive cases using real-time PCR analyses. Representative pictures of real-time PCR and gel electrophoresis to identify EBV strains and variants are depicted in Fig. 2A–H. Overall, the most common EBV strain detected in this study was type A (52.1 %), followed by type B (32.1 %) and type AB (15.8 %) (Table 3). EBV type A was more predominant than type B in every group. Participants in the NOM group possessed a higher percentage of EBV type A (72.0 %) compared to those in the

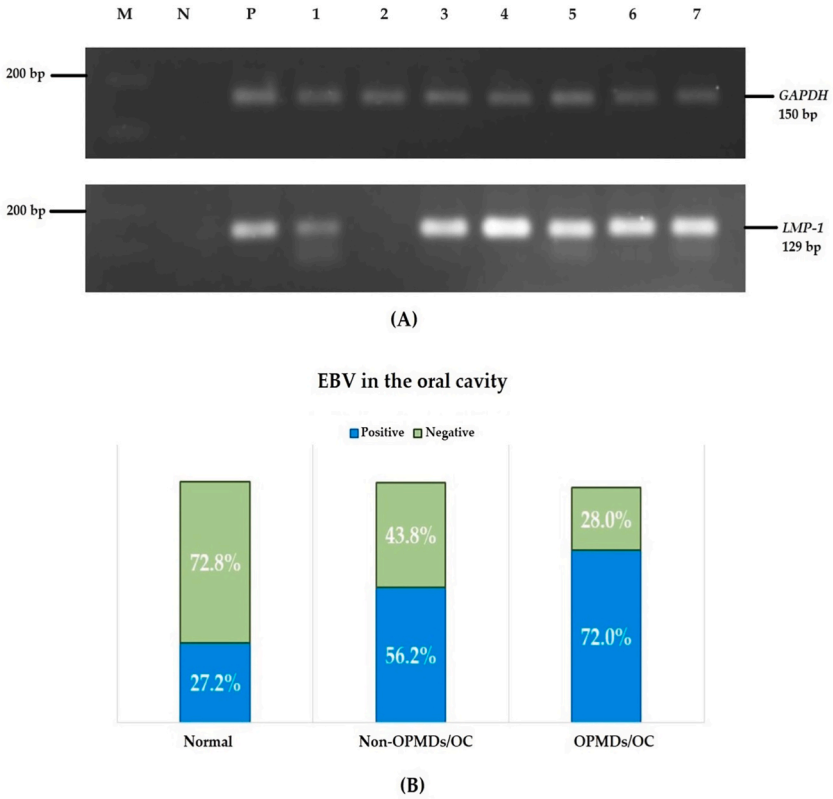

Fig. 1. Representative pictures of gel electrophoresis showing PCR products (A): *GAPDH* and *LMP1* gene. Lane M: 100 bp DNA ladder, Lane N: Negative control, Lane P: Positive control, Lanes 1: *LMP1* positive samples diagnosed with OSCC, Lane 2: *LMP1* negative sample from normal oral mucosa. Lanes 3 and 4: *LMP1* positive sample diagnosed with mild epithelial dysplasia, Lane 5: *LMP1* positive sample diagnosed with fibrous hyperplasia (fibroma), Lanes 6 and 7: *LMP1* positive samples diagnosed with oral lichen planus. The original pictures of gels of PCR products for *GAPDH* and *LMP1* can be visualized in supplementary files named GAPDH control and EBV PCR analysis, respectively. (B) Prevalence of EBV in the oral cavity among various oral mucosal conditions.

Table 2
Demographic data and risk factors of oral cancer.

Variable	EBV			
	Negative	**Positive**	**Total n (%)**	
	(n = 150)	**(n = 165)**		***p*-value**
	n (%)	**n (%)**		
Demographic data				
Age in years (n=315)				
40-65	92 (54.4)	77 (45.6)	169 (53.7)	**0.009**
>65	58 (39.7)	88 (60.3)	146 (46.3)	
Median (Min-Max)	62 (40–87)	67 (40–88)	64 (40–88)	**0.002**
Sex (n=315)				
Female	120 (52.4)	109 (47.6)	229 (72.7)	**0.006**
Male	30 (34.9)	56 (65.1)	86 (27.3)	
Oral mucosal condition (n=315)				
Normal	67 (72.8)	25 (27.2)	92 (29.2)	**<0.001**
Non-OPMDs/OC	57 (43.8)	73 (56.2)	130 (41.3)	
OPMDs/OC	26 (28.0)	67 (72.0)	93 (29.5)	
Risk factors of oral cancer				
Tobacco (n=314 [a])				
Non-smoker	131 (51.4)	124 (48.6)	255 (81.2)	**0.008**
Current or ever-smoker	19 (32.2)	40 (67.8)	59 (18.8)	
Smokeless tobacco (n=314 [a])				
Non-user	140 (48.8)	147 (51.2)	287 (91.4)	0.24
Current or ever-user	10 (37.0)	17 (63.0)	27 (8.6)	
Alcohol drinking (n=314 [a])				
Non-drinker	122 (50.6)	119 (49.4)	241(76.8)	0.07
Current or ever-drinker	28 (38.4)	45 (61.6)	73 (23.2)	
Betel quid chewing (n=315)				
Non-chewer	111 (52.4)	101 (47.6)	212 (67.3)	**0.02**
Current or ever-chewer	39 (37.9)	64 (62.1)	103 (32.7)	
Working in sunlight (n=314 [a])				
No	86 (55.1)	70 (44.9)	156 (49.7)	**0.009**
Yes	64 (40.5)	94 (59.5)	158 (50.3)	

Bold indicates significant difference at p-value< 0.05 [a] Missing data = 1.

non-OPMDs/OC (54.8 %) and the OPMDs/OC (41.8 %) groups. EBV type B was observed in 24.0 % in the NOM group, 31.5 % in the non-OPMDs/OC group and 35.8 % in the OPMDs/OC group. Both EBV type A and B were mostly found in the OPMDs/OC group (22.4 %) (Table 3)

Regarding EBV variants (Fig. 2E–H), the majority of EBV-positive participants (64.8 %) had wt-LMP1, whereas 33.3 % had del-LMP1 variant (Table 3). The prevalence of del-LMP1 was highest in the non-OPMDs/OC group (38.4 %), while the prevalence of wt-LMP1 was highest in the NOM group (80.0 %). Three samples out of 165 DNA samples displayed both variants (two in the non-OPMDs/OC group and one in the OPMDs/OC group).

3.4. Association of EBV and risk factors of OC

To examine factors that independently affected the presence of EBV in the oral cavity, a logistic regression analysis was performed. Age, sex, oral mucosal conditions, and risk factors for OC were used as independent variables and EBV positivity was used as the dependent variable (Table 4). The result indicated that there was no association between risk factors for OC and EBV. Interestingly, the presence of EBV in oral cavity was associated with oral mucosal conditions. Participants in the non-OPMDs/OC and the OPMDs/OC groups showed 2.39 (95 % CI: 1.26–4.53) and 4.48 times (95 % CI: 2.18–9.22) increased risk to be positive for EBV, respectively.

4. Discussion

We investigated the prevalence of EBV in the oral cavity of participants with NOM, non-OPMDs/OC, and OPMDs/OC and found that the prevalence of EBV was 27.2 %, 56.2 % and 72.0 %, respectively (Fig. 1B). Although EBV was detected in both OPMDs and OC, it was also found in healthy oral mucosa. Hence the oral cavity can be considered as EBV reservoir [13,14]. The prevalence of EBV in the normal oral cavity ranged from 4 to 40.4 % worldwide [15–17]. In our study, overall EBV prevalence in NOM was 27.2 %, consistent with previous studies (Fig. 1B) [15–17]. In the central region of Thailand, the prevalence of EBV was 26.4 % in NOM specimens using immunohistochemistry [18], whereas in the northeastern Thailand, Acharya et al. reported 18.08 % of EBV-positivity in non-cancer control using oral exfoliated cells with nested PCR [19]. Both studies have shown a lower rate of EBV in the normal oral cavity compared to our study (27.2 %). The disparities could be attributed to the region's diverse population, various types of samples, and detection methods.

To the best of our knowledge, we are the first group to explore EBV frequency in the non-OPMDs/OC, with 56.2 % of the subjects

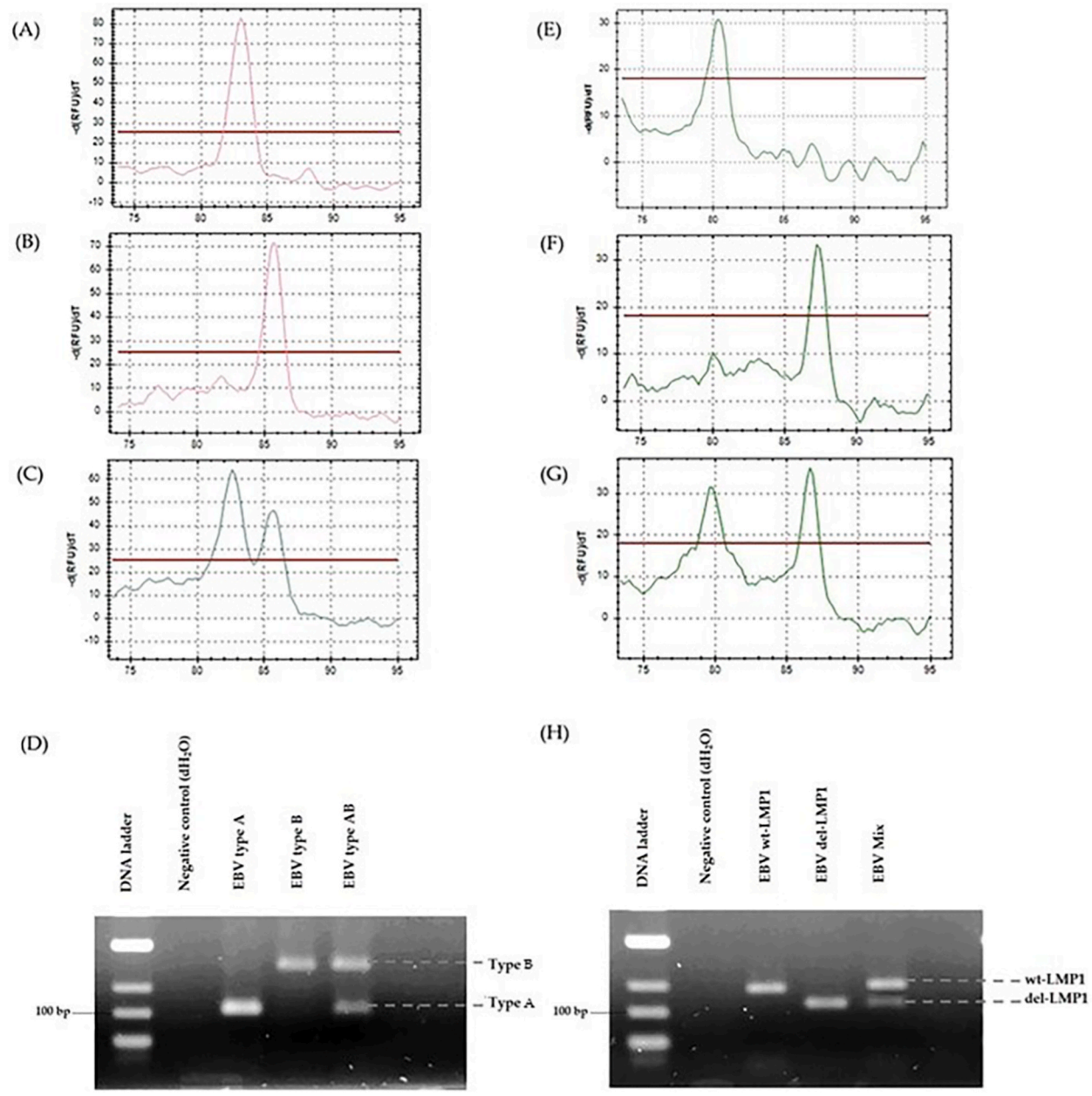

Fig. 2. Representative pictures of melt curve and gel electrophoresis of qPCR products. EBV strains and variants were identified based on melting curves exhibiting the varied dissociation temperature. (A)–(C) represented EBV type A, B and AB, respectively. These findings corresponded to gel electrophoresis result (D). (E)–(G) showed EBV wild-type *LMP1*, 30-bp deletion at *LMP1* and mixed type. The EBV mix (G) melt curve was positioned at the same melting temperature as shown in (E) and (F). These findings conformed with the gel electrophoresis observation (H). The original picture of gel illustrating the PCR products to identify strains and variants can be visualized in a supplementary file named strain and variant PCR analyses.

testing positive for EBV (Fig. 1B). It is important to note that the non-OPMDs/OC group does not show the same EBV prevalence as the OPMDs/OC group, while having a higher EBV frequency compared to the NOM group. This observation may be attributable to changes in the oral environment that resulted in a greater EBV reservoir in the oral cavity in comparison to NOM.

Previous investigations on EBV prevalence in OPMDs and OC produced disparate results, which appeared to be dependent on the type of samples, sample collection procedures, EBV detection techniques, geographic locations, ethnic variations, and the effects of risk factors for OC. The prevalence of EBV in OSCC ranged from 0 % to 100 %, while OPMDs ranged from 26.1 to 97.4 % worldwide [13,15, 19–21]. In our study, 72.0 % of OPMDs/OC group was tested positive for EBV (Fig. 1B). A comparable result done by Kikuchi et al. revealed that 72.2 % of formalin-fixed, paraffin-embedded (FFPE) tissues of OPMDs were positive for EBV using *LMP1* primers with

Table 3
The distribution of EBV strains based on *EBNA3C* and EBV variants based on *LMP1* in EBV positive samples (n = 165).

Oral mucosal condition	EBV strains			*LMP1* variants		
	Type A	Type B	Type AB	wt-LMP1	del-LMP1	Mix
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Normal (n = 25)	18 (72.0)	6 (24.0)	1 (4.0)	20 (80.0)	5 (20.0)	0 (0)
Non-OPMDs/OC (n = 73)	40 (54.8)	23 (31.5)	10 (13.7)	43 (58.9)	28 (38.4)	2 (2.7)
OPMDs/OC (n = 67)	28 (41.8)	24 (35.8)	15 (22.4)	44 (65.7)	22 (32.8)	1 (1.5)
Total (n=165)	86 (52.1)	53 (32.1)	26 (15.8)	107 (64.8)	55 (33.3)	3 (1.9)

Table 4
Association of EBV and risk factors of oral cancer (n = 314).

Variable	Adjusted odd ratio (95 % CI)	*p*-value
Age in years		
≤65 (n = 168)	Ref.	
>65 (n = 146)	1.20 (0.71–2.05)	0.5
Sex		
Female (n = 228)	Ref.	
Male (n = 86)	1.79 (0.84–3.82)	0.13
Oral mucosal condition		
Normal (n = 92)	Ref.	
Non-OPMDs/OC (n = 130)	2.39 (1.26–4.53)	**0.008**
OPMDs/OC (n = 92)	4.48 (2.18–9.22)	**<0.001**
Tobacco		
Non-smoker (n = 255)	Ref.	
Current or ever-smoker (n = 59)	1.31 (0.53–3.22)	0.55
Smokeless tobacco		
Non-smoker (n = 287)	Ref.	
Current or ever-smoker (n = 27)	0.93 (0.38–2.31)	0.88
Alcohol drinking		
Non-drinker (n = 241)	Ref.	
Current or ever-drinker (n = 73)	0.97 (0.49–1.89)	0.92
Betel quid chewing		
Non-chewer (n = 212)	Ref.	
Current or ever-chewer (n = 102)	1.67 (0.89–3.12)	0.11
Working in sunlight		
No (n = 156)	Ref.	0.21
Yes (n = 158)	1.39 (0.83–2.32)	

Bold indicates significant difference at p-value<0.05.

PCR analysis [13]. Cruz et al. found *LMP1* expression in all OSCC and 77.8 % of OPMDs using frozen block tissue biopsy samples and PCR [20]. Another study using qPCR found that the *LMP1* was present in 58.3 % and 41.7 % of the saliva of patients with OSCC and OPMDs, respectively [15].

Regarding EBV in OPMDs/OC in Thailand, Acharya et al. used nested PCR to show that *LMP1* was identifiable in 32.5 % of oral exfoliated cells from OSCC patients in the northeastern region [19]. By contrast, another study in the northern area was unable to detect EBER in OSCC FFPE specimens [21]. In the central region of Thailand, Rahman et al. found that LMP1 was overexpressed in 59.6 % of OSCC FFPE specimens using immunohistochemistry, whereas oral leukoplakia with and without dysplasia demonstrated EBV-positivity at the rates of 34.15 % and 28.03 %, respectively [18].

For further analysis, identification of strains and variants of EBV was conducted in EBV positive samples (Fig. 2A–H). This study is the first to identify EBV strains and variants in the oral cavity among Thai population. A polymorphism in the *EBNA* gene has long been known to distinguish between two EBV strains. The difference in host cell transformation ability was observed due to the divergence in the *EBNA* gene. For example, EBV type A has a greater potential to transform B-cells than type B and is predominantly observed in Asia and Western countries. In contrast, EBV type B was obtained from Burkitt's lymphoma cell, which is prevalent in the African sub-Sahara endemic area [8]. Interestingly, both EBV type A and B can be present in healthy adults [22,23].

The overall prevalence of EBV type A, B, and AB in our study was 52.1 %, 32.1 %, and 15.8 %, respectively (Table 3). Representative pictures of real-time PCR analysis to identify EBV strains are depicted in Fig. 2A–D. EBV type A was found to be predominant in all groups but seemed to be higher in the NOM group whereas EBV type B was more often found in the participants with oral lesions including those with OPMDs/OC and non-OPMDs/OC (Table 3). Interestingly, the participants in the OPMDs/OC group exhibited the

highest percentage of EBV type A and B positivity (22.4 %) compared to those in the non-OPMDs/OC (13.7 %) and the NOM (4.0 %) groups. The data in this present study were similar to some other studies in that EBV type A was more mainly found in every group. According to a study conducted in Japan, PCR analysis of *Bam*HI–*F*, *EBNA2* and *LMP1* genes was performed in 95 participants with OSCC and it was found that 51.6 % of participants were positive for EBV. Of these, 69.4 % had EBV type A, 14.3 % had EBV type B and 16.3 % had both types [24]. In another study conducted in Portugal, EBV type A alone was present in 97.6 %, 85.7 % and 79.5 % in the peripheral blood of participants in the control, the head and neck squamous cell carcinoma (HNSCC) and the NPC groups, respectively [25]. Furthermore, previous studies reported that EBV type A was more predominant than type B in NPC, normal stomach and gastric lesions [9,10]. These results are consistent to our study where EBV type A was more often found in all groups. However, the percentage of EBV type B in the OPMDs/OC (35.8 %) and the non-OPMDs/OC (31.5 %) groups was higher than other studies which reported the frequency ranging from 0 to 15 % in NPC, OSCC and HNSCC [9,10,24,25].

The most frequent *LMP1* variant is a 30-bp deletion of *LMP1* in the C-terminus first detected in NPC patients from Southern China [26]. The 30-bp deletion results in a loss of 10 amino acids (residues 346–355) involved in the transformation effector site 2 (TES2), leading to a more oncogenic variant [27]. A recent meta-analysis evaluated the association of this variant with NPC development and showed that the association is observed in Asian populations while no association is observed for European and North African countries [28]. In our study, 20.0 % of participants with NOM had del-LMP1 in their oral cavities. The higher del-LMP1 prevalence was observed in the OPMDs/OC (32.8 %) and the non-OPMDs/OC (38.4 %) groups (Table 3). Comparing this present study to the study in Portugal, del-LMP1 was detected in 63.2 % in the peripheral blood of participants with HNSCC compared to 26.2 % in control participants [25]. In Thailand, the prevalence of del-LMP1 in the peripheral blood of participants with NPC was 58.7 % compared to 36.4 % in controls [9]. A recent PCR study also conducted in Thailand reported that the prevalence of del-LMP1 were 36.4 %, 22.2 % and 12.5 % in FFPE tissues of gastric carcinoma, intestinal metaplasia and normal stomach, respectively. Although statistical analysis regarding the association of del-LMP1 and OPMDs/OC was not performed in this study due to low number of del-LMP1 positive cases, there is a trend of increasing prevalence of this variant in the oral mucosal lesion, compared to NOM.

We further assessed the effect of other common risk factors for OC including tobacco smoking, use of smokeless tobacco, alcohol intake, betel quid chewing and chronic sun exposure on EBV frequency (Table 4). Using a logistic regression model, no association of EBV prevalence with risk factors for OC was found. Similarly, Nasher et al. also found no substantial OSCC risk in subjects with positive EBV using buccal cells and biopsies with qPCR technique [29]. Our result is also consistent with other investigations, reporting that smoking tobacco and alcohol drinking were not risk factors for the presence of EBV in the oral cavity [16,17,20]. A similar correlation between EBV and such habits was reported by Acharya et al., nevertheless, betel quid chewing seems to increase EBV prevalence [19]. Interestingly, association of EBV prevalence and oral mucosal condition was observed in our study with adjusted odd ratio of 2.39 (95 % CI: 1.26–4.53) and 4.48 times (95%CI: 2.18–9.22) for the non-OPMDs/OC and the OPMDs/OC groups, respectively. This conclusion is consistent with the findings of OSCC research in that the presence of EBV was associated with the occurrence of OSCC and OPMDs [20].

Although some interesting results were appreciated, we acknowledged the limitation of this study in that the number of participants in each group was low and the number of EBV positive samples was unequal among groups. In addition, stringent follow up may be required, especially in the group of participants with OPMDs and EBV positivity to prevent the transformation of these oral lesions to OC.

5. Conclusions

The prevalence of EBV in this group of Thai participants with OPMDs/OC was considerably greater than in other oral conditions. EBV type A was found to be predominant than type B in all groups. Although wt-LMP1 variant was more often found in all groups, higher percentage of del-LMP1 was found in the groups with oral mucosal lesions.

Ethics statement

This study was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COA. No.MU-DT/PY-IRB 2019/050.3107 and COA.No.MU-DT/PY-IRB 2019/041.0307). Each subject acknowledged the protocol verbally and in writing and then written informed consent forms were signed. The ethical guidelines of the Declaration of Helsinki were followed throughout the study.

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Data availability statement

The data associated with this study have not been deposited into a publicly available repository due the confidentiality of the participants but are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Pasinee Vorakulpipat: Writing – original draft, Investigation, Formal analysis, Data curation. **Nakarin Kitkumthorn:** Resources, Methodology. **Puangwan Lapthanasupkul:** Resources, Methodology. **Dulyapong Rungraungrayabkul:** Writing – review & editing, Data curation, Formal analysis, Conceptualization, Methodology. **Boworn Klongnoi:** Resources, Funding acquisition. **Siribang-on Piboonniyom Khovidhunkit:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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