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ORIGINAL ARTICLE

Epstein–Barr virus infection in B-cell Non-Hodgkin's Lymphomas of the Oral and Maxillofacial Region: Is there any evidence?

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Abstract

Introduction: Epstein–Barr virus (EBV) might be an aetiological agent involved in the pathogenesis of certain Non-Hodgkin's Lymphomas (NHLs). EBV infection has been diagnosed by serologic testing within the tumour biopsies of patients with NHL. However, the association between EBV and NHL is inconsistent with a preference for certain anatomic sites, histologic subtypes and immunosuppressed patients. The objective of this study was to characterise the B-cell NHLs of the oral cavity and maxillofacial region using histological and immunophenotypical techniques and to determine its association with EBV infection. *Materials and Methods:* This was a descriptive cross-sectional study that included 14 cases of B-cell NHLs of the oral cavity and maxillofacial region. The haematopoietic and lymphoid tissue tumours classification of WHO was used to categorize the cases. In-situ hybridisation for EBV–encoded RNA was performed to confirm the EBV infection. *Results:* The average age of the patients included in the study was found to be 48.8 ± 23 years with a higher female to male ratio (1.3:1). Our study suggested that diffuse large B-cell lymphomas (DLBCLs) and Burkitt's lymphomas (BLs) constitute the predominant subtypes of lymphomas affecting the oral cavity and maxillofacial regions. *Conclusion:* The findings from our study support the view that at least a relatively smaller proportion of B-cell NHLs that occur in the oral cavity and maxillofacial region do not have a pathogenic association with EBV.

Keywords: B-cell NHL, Epstein–Barr virus, in-situ hybridisation, maxillofacial region, oral cavity

INTRODUCTION

Non-Hodgkin's lymphomas (NHLs) comprise of a variety of malignant lymphoproliferative disorders that are defined by lymphocytic clonal expansion observed at different stages of ontogenetic development. NHLs involving the jaws and oral cavity constitute around 2% of all NHLs.[1] Among all anatomic regions, B-cell lymphomas account for up to 80-85% of NHLs. Most oral cavity lymphomas are of B-cell type which ranges from 41-100% and the diffuse large B-cell lymphoma (DLBCL) is the most frequent histologic type.[2] A lymphoma that arises in the oral cavity is often a part of the secondary malignant proliferation, which affects the regional nodes, or it could be a primary extranodal disorder restricted to the oral cavity or jaws.[3]

Around 5% of the head and neck cancers are NHLs which widely varies in appearance and might resemble Hodgkin's disease.[4] NHL includes a variety of subtypes based on clinico-histopathologic and immunologic presentation and is related to diverse aetiologies such as Epstein–Barr virus (EBV) infection, genetic susceptibility and immunocompromised state.[3] Available literature based on immuno-histochemical staining revealed that lymphomas of oral cavity are mainly of B-cell type. On the other hand, most nasal and paranasal sinus lymphomas constituted a T-cell type. This suggests an association of B-cell lymphomas with oral cavity whereas; nasal cavity and para-nasal sinuses had predominance of T-cell lymphomas.[5]

EBV is a DNA virus which belongs to the human herpes virus family. To date there is inconsistent evidence for the pathologic association of EBV with NHLs. The presence of EBV infection among general population

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speculates that its detection in tumours could also be incidental and it might involve in carcinogenicity of some NHL types. After primary infection, the EBV may remain within the resting B-cells and cytotoxic T-cells (CTLs) in an asymptomatic latent state in the host for the lifetime[6]. B-cells act as reservoir of EBV resulting in a carrier state which may sometimes be transformed into malignant lymphomas.[7]

To date several studies have reported lymphomas in the oral cavity and maxillofacial (OCM) region as it is a natural site of EBV replication. Epstein et al[8] reported the largest series with 361 cases of lymphomas in the oral cavity and para-oral region. Better understanding of the etiologic involvement of EBV in the development of lymphoma may facilitate the inception of advanced targeted therapies against specific EBV viral antigens.[6] However, there is paucity of available information with respect to EBV infection and oral lymphomas in Malaysia. Herein, the present study aims to characterise the B-cell NHLs of the OCM region using histological and immunophenotypical methods and determine the pathogenic association between B-cell NHL and EBV infection.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted that included 14 cases of B-cell NHL of the OCM region. The Medical Ethics Committee at Faculty of Dentistry, University of Malaya approved this study [Ethics DF OS1421/0095(P)].

All lymphoma biopsy specimens diagnosed as B-cell lymphomas from the year 1981 to 2015 were retrospectively identified from the records of the Oral Pathology Diagnostic and Research Laboratory, at the Faculty of Dentistry, University of Malaya. The primary inclusion criteria were (a) diagnosis of B cell lymphoma whether primary, recurrent, or secondary as determined by immunopositivity of tumour cells for CD20 and immunonegativity of tumour cells for CD3/CD45RO (b) Cases with complete demographic data (c) involvement of an oral site or maxillofacial region and (d) availability of formalin fixed paraffin embedded tissue blocks with adequate tissue preservation.

The WHO classification for tumours of haematopoietic and lymphoid tissues was used to categorise the cases based on morphology and immunologic findings.[9] Formalin fixed paraffin embedded tissue sections were stained with Haematoxylin and Eosin (H&E) stain.

Immunohistochemical staining

Immunoperoxidase Envision technique, Dako REAL™ EnVision™ Detection System using a set of monoclonal antibodies to CD45 (Dako, M0701, dilution 1:200), CD20 (Dako, M0755, dilution 1:1000) , CD19 (Dako, M7296, dilution 1:50), CD10 (Dako, M7308, dilution 1:100), CD3 (Dako, A0452, dilution 1:300), Mum1 (Dako, M7259, dilution 1:25), PAX5 (Dako, AB55492, dilution 1:50), CD30 (Dako, M0751, dilution 1:300), CD15 (Dako, M3631, dilution 1:200), CD5 (NovoCastra, NCL-CD5-4C7, dilution 1:50), CD23 (NovoCastra, NCL-CD23-1B12, dilution 1:20), BCL-2 (Dako, M0887, dilution 1:200), Cyclin D1 (ThermoScientific, RM 9104-s, dilution 1:50) and TdT (NovoCastra, NCL-TdT-339, dilution 1:50) antigens was used to assess the immunohistochemical staining in tissue sections. The source of primary antibodies and their dilution is listed in parentheses. The immunohistochemical staining was reviewed and evaluated by Dr. Laila M Abdelrahim and Dr. Thomas George Kallarakkal. The results were verified by Dr. Peh Suat Cheng. All the evaluators were blinded from the diagnosis.

In-situ hybridisation for EBV

The detection of Epstein-Barr encoded RNA (EBER) in tissues was performed by in-situ hybridisation (ISH) with a fluorescein conjugated peptic nucleic acid (PNA) probe specific for EBER (PB0589, PNA ISH Detection Kit; Leica). For positive control, a confirmed EBV-positive tissue section was used which was run in parallel with all analyses that were tested as per the manufacturer's instructions. There was no specific step included for RNA preservation. A positive EBER-ISH result was characterised by the presence of brown staining of the nuclei of the tumour cells. The in-situ hybridisation staining was evaluated by Dr. Peh Suat Cheng who was blinded from the histopathological diagnoses of the cases.

RESULTS

A total of 14 patients diagnosed with B-cell NHL of the OCM region were included in the present analysis (Table 1). More than half (57.1%) of them were females (female to male ratio: 1.3:1). The average age of the subjects was 48.8±23 years (median age, 51 years). The most frequently affected ethnic groups were Malays (42.8%), Chinese (35.7%) and other nationalities (21.4%). The primary clinical symptom identified in almost all the patients was a progressively

TABLE 1: Presentation of demographics, site and types of lymphoma in cases of B-cell NHL

No.	Age (yrs)	Gender	Ethnicity	Site	Types of lymphoma
1	65	Female	Others	Submandibular region	DLBCL
2	64	Male	Malay	Palatal region	MCL
3	30	Male	Malay	Gingiva	BL
4	49	Female	Chinese	Preauricular region	FL
5	49	Female	Malay	Submandibular region	DLBCL
6	90	Male	Malay	Soft palate	DLBCL
7	11	Female	Chinese	Buccal mucosa	BL
8	69	Female	Others	Submandibular region	DLBCL
9	65	Female	Malay	Submandibular region	DLBCL
10	44	Female	Chinese	Buccal mucosa	BL
11	11	Male	Chinese	Lingual mucosa	BL
12	53	Male	Indian	Buccal mucosa	FL
13	15	Female	Chinese	Buccal mucosa	BL
14	68	Male	Malay	Soft palate	CLL

DLBCL: diffuse large B-cell lymphoma; BL: Burkitt's lymphoma; FL: follicular lymphoma

increasing, diffuse swelling that was not tender to palpation with normal surrounding skin and mucosa. An ulcerative growth was the chief complaint in one of the patients.

The predominant histologic subtypes of lymphomas affecting the OCM region were DLBCL (35.7%) and BL (35.7%) followed by follicular lymphoma (FL: 14%). The average age of the patients diagnosed with DLBCL and BL was 67.6 years and 22.2 years, respectively. Majority of the DLBCL (60%) cases were observed among the Malays whereas BL was identified more commonly among the Chinese (80%) ethnicity. Most of the DLBCL cases were identified in the submandibular region while, BL involved various subsites such as the buccal mucosa, lingual mucosa and mandibular bone.

Five of our cases were diagnosed as DLBCL and four of them (80%) occurred in females. 60% of all DLBCL cases were classified as non-germinal center B-cell (non-GCB) subgroup (positive to MUM1 and negative to CD10). Cases diagnosed as DLBCL showed a diffuse, monotonous proliferation of round cells with enlarged nuclei and a limited amount of cytoplasm resembling lymphoid cells (Fig. 1).

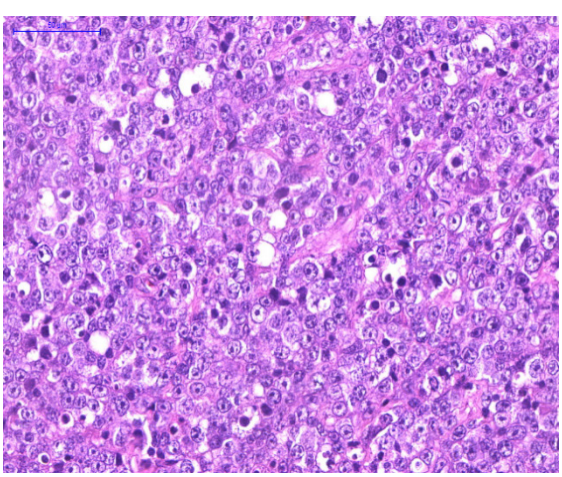

FIG. 1: Representative section of DLBCL showing diffuse large cells with scanty cytoplasm and large nuclei (H & E Stain, Magnification 40X).

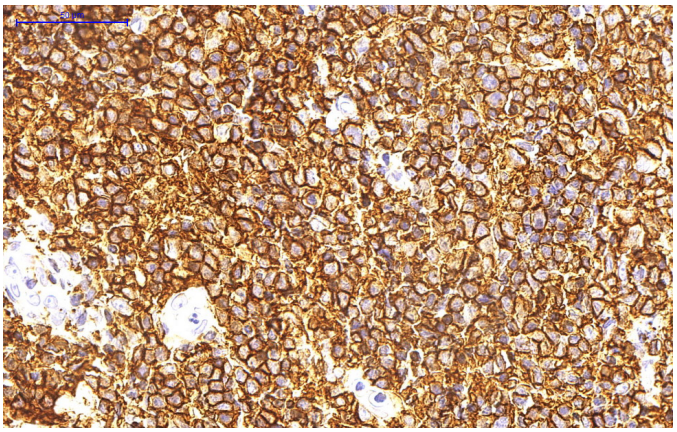

FIG. 2: Diffuse positivity of tumour cells (DLBCLs) for CD20 (Magnification 40x).

All cases of DLBCL showed strong membranous positivity to CD20 (Fig. 2) and CD19. A strong nuclear staining with PAX5 was observed.

Three out of five cases of BL (60%) occurred in very young patients (≤15 years) and accounted for all children in the study. For BL cases, the microscopic examination of tumour showed densely packed, uniform lymphoid cells of medium size with interspersed histiocytes presenting the classical starry sky appearance indicative of BL (Fig. 3). Mitosis was abundant in these cases. All the cases of BL showed strong CD10 membranous positivity, in addition to CD20 (Fig. 4), and CD19. None of the samples were found to be positive for TdT and Bcl-2 staining.

EBV infection was assayed by EBER in-situ hybridisation. A confirmed EBV-positive section of nasopharyngeal carcinoma served as the positive control (Fig. 5A). None of our samples showed expression of EBER in the nucleus (Fig. 5B).

DISCUSSION

Demographics and clinical characteristics

The present study reported 14 cases of B-cell NHLs in the OCM region. We observed a female predominance among our cases. The reported observation of variation in gender distribution among the various studies may be attributed to racial differences, genetic and environmental factors and differences in the sample size.[4] The mean age of our samples was 48 years and the

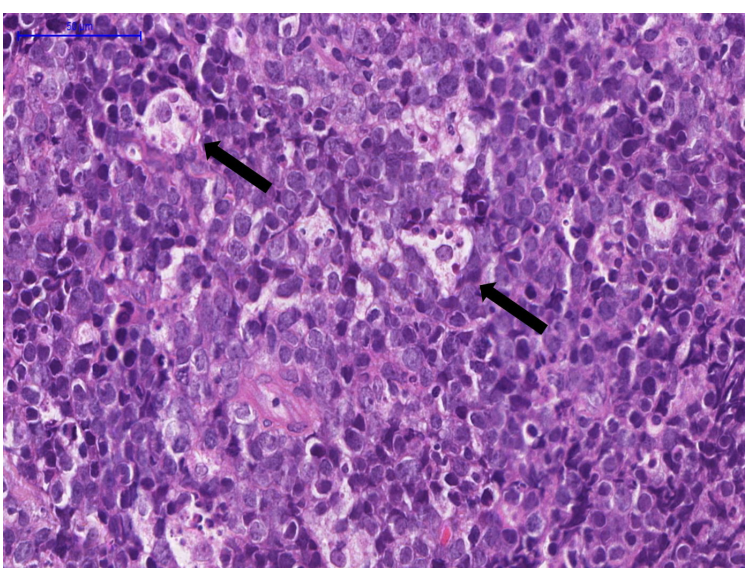

FIG. 3: Representative section of BL showing small round cells with scanty cytoplasm interspersed with histiocytes (Arrow) (H & E Stain, Magnification 40X).

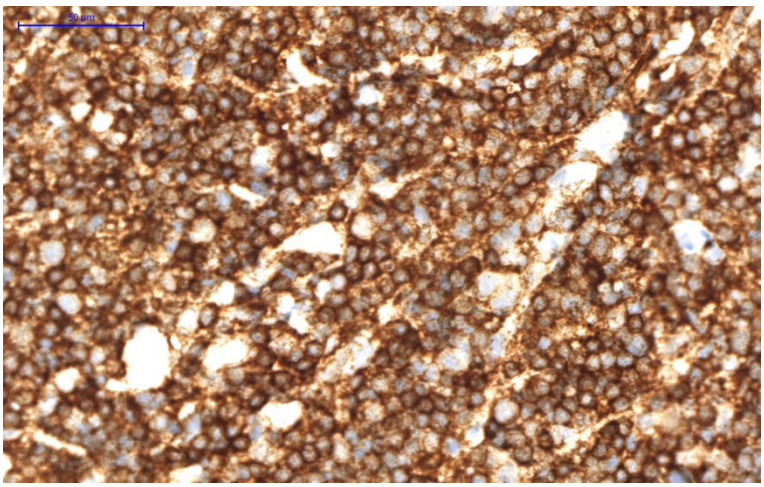

FIG. 4: Diffuse positivity of tumour cells (BL) for CD10 (Magnification 40x).

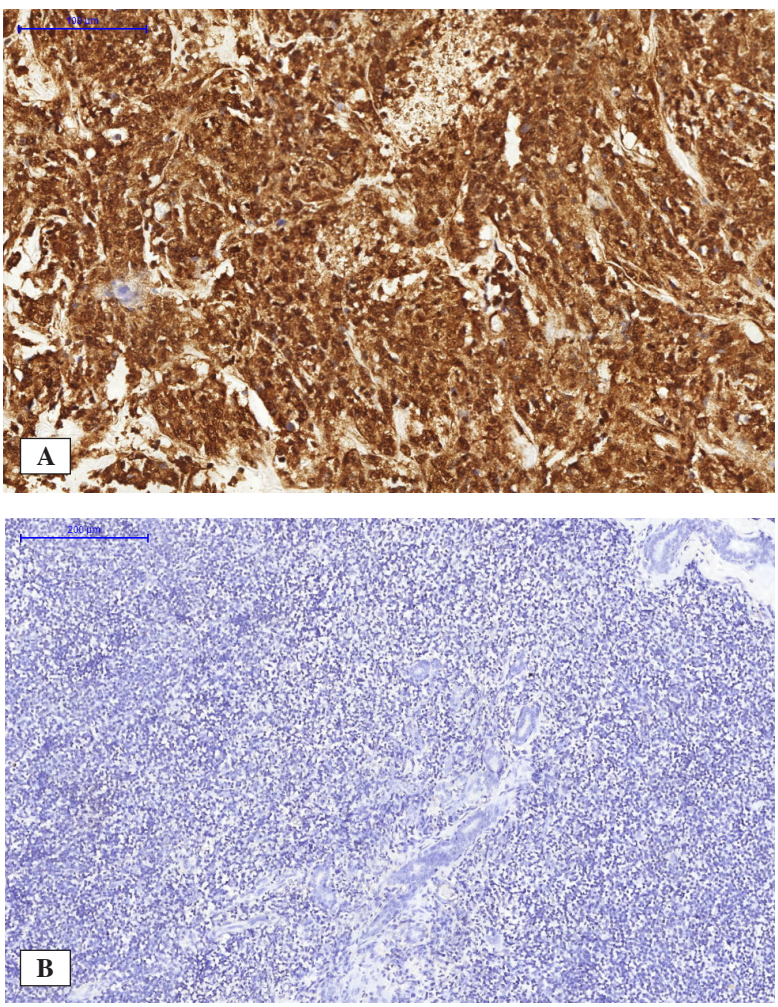

FIG. 5: EBER positive nuclei in a positive control slide of nasopharyngeal carcinoma (A) (Magnification 20x); Negative staining for EBER in our case (B) (Magnification 10x).

median age was 51 years. There seems to be a worldwide tendency that the incidence of head and neck NHLs increases with advancing age[3]. Our findings are in agreement with published reports even though our study was limited only to B-cell NHLs. The submandibular region and the buccal mucosa were the most frequently affected sites in our study. A limited number of reports describe the location of intraoral NHLs which include the palate, tongue, and the cheek.[10] Based on these previous reports and the findings from our study, it is observed that there is no specific site predilection for B-cell NHLs in the OCM region.

Classification of B-cell NHLs

In our study, DLBCLs (35%) and BLs (35%) were the most common histologic subtypes among all B-cell NHLs followed by FL (14%). DLBCLs preferably occur in the head and neck region[11]. MUM1 protein expression is correlated with adverse prognosis in DLBCL according to most but not all reports.[12] Majority of our cases of BL occurred in children. This was in concordance with a previous study from Malaysia on childhood malignancies which suggested BL as the second most frequent subtype of lymphoma among children.[13] All cases of BL in our series showed an absence of staining to bcl-2 and TdT. TdT staining was used to differentiate BL from lymphoblastic lymphoma (LL) as both are characterised histologically by having a starry sky appearance. In our study, the percentage of cases diagnosed as FL was 14%. The lowest rates of FL have been reported among Asian population.[14] The small sample size in our study may preclude us from drawing any plausible conclusions regarding the prevalence rates of FL in this region. All our FL cases showed a positive staining for CD10 and Bcl-2.

The Bcl-2 and CD10 markers were considered based on their presumed involvement in the development of FL. The cytogenetic detection of reciprocal translocation t(14;18), of Bcl-2 oncogene and immunoglobulin heavy chain joining region is the characteristic feature of FL.[15]

We reported a patient (7%) with mantle cell lymphoma (MCL). The tumour cells were positive for CD20, Bcl-2, CD5, CD23 and cyclin D1 but negative for CD10, which helped to discriminate it from chronic lymphocytic lymphoma (CLL) and FL. In this study, we reported one case of CLL (7%). A specific immunophenotype was seen in our case of CLL which showed pan-B cell, CD5 and CD23 markers positivity. Interestingly, CD5 is a marker for T cells which for unexplained reasons is expressed on CLL tumour cells.

Incidence and detection of EBV infection

In the south East Asian region, EBV infection is commonly seen in the oral cavity. This may be attributed partially to the dietary practices in the Asian culture where mothers pre-masticate food to feed the young children as well as the use of same chopsticks. Therefore, the exchange of saliva among household members could be a possible reason for the transmission of EBV.[16]

There is a marked variation in the incidence and frequency of EBV-associated malignancies worldwide. A higher association of BL with EBV has been reported in studies mainly from Equatorial Africa. In comparison, only few cases of BL have been identified with EBV from other regions. The observed demographic association of EBV-related malignancies remains uncertain. The possible explanation could be attributed to the host genetic variability, viral characteristics and environmental factors. Marked sequence variability has been identified in EBV genome at different loci but still the role of genetic heterogeneity in the tumour pathogenesis needs further investigation.[17]

An earlier study detected frequent association of EBV-related BL among Chinese ethnicity which accounted for 100% of cases.[18] It has been identified that EBV infection is also associated with DLBCL; notably among the older age group (>50 years). An Asian study which focused on this age group reported the frequency of EBV-related DLBCL to be 8-10%. The proposed hypothesis for such an association is the inheritance of defective immune mechanism against EBV infection during the aging process. In contrast to Asian population, the incidence of DLBCL in the advanced age is relatively lower among elderly population from the western countries. Exceptionally, the prevalence rate among Mexican elderly population (7%) is comparable to the reported frequency from the Asian region.[19] Contrary, to earlier reports, a report from northern China showed a frequency of EBV-positivity in only 8 of 212 patients older than 50 years with DLBCL.[20] It has been suggested that the detection rate of EBV-positive DLBCL among the overall as well as selected elderly population is particularly dependent on the chosen threshold for EBR positivity making the geographic distribution controversial.[21]

EBV infection is highly associated with lymphomas in immuno-compromised patients. Leong et al.,[2] demonstrated that all of the immuno-suppression-related oral NHLs were EBV-positive, while the rate of EBV positivity in the presumed immuno-competent NHLs patients was only 9%. Immuno-compromised patients primarily have impaired lymphocyte cytotoxic pathway or T-cell defect that also involves lack of interaction between B-cells and T-cells. The high association of EBV infection with lymphoma in immunocompromised patients may be attributed to these genetic defects.[21]

Bahnassy et al[22] detected the EBV genome in 70-90% of head and neck extranodal NHL samples by EBER in-situ hybridisation and PCR in Egypt. It has been suggested that the detection rate of EBV infection in NHLs is influenced by various factors such as the patient's immune status, histological subtypes of the tumour, the anatomical site of the tumour, and the sensitivity of the detection method. In-situ hybridisation (ISH), Polymerase Chain Reaction (PCR), and southern blotting are highly sensitive methods for detection of EBV infection. Moreover, RNA-ISH is the gold standard procedure for the diagnosis of EBV infection which allows detection of infected cell types.[23]

Qi et al.,[24] compared different diagnostic methods for EBV infection using immunohistochemistry to detect latent membrane protein 1, ISH for EBER-1 and PCR for EBV genome. The authors found PCR to be the most sensitive method but it lacked definitive information about cellular localization which is provided by other methods. ISH technique is quicker and simpler than the other methods used for the identification of EBV. In addition, use of biotinylated probes for paraffin-embedded tissues is safer to handle the infectious material and use of preserved tissue samples that is not feasible with DNA extraction method.[25] Peh et al.,[26] reported a strong association of EBV infection with T-cell NHLs but not B-cell NHLs of the upper aero-digestive tract in non-immunocompromised Malaysian subjects, despite the fact that B-cells are considered as carriers of EBV. Similarly, EBV infection was not detected by ISH in any of our cases.

In conclusion, this study revealed that B-cell NHLs which occur in proximity to sites of natural EBV replication do not preferentially harbor EBV in either its latent or replicative form.

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