Original article

Application of Fine Needle Aspiration Biopsy and Immunohistochemical Diagnostic Markers in the Identification of Oral Primary B-cell Lymphoma

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Abstract

Objectives: The current research aimed to evaluate the role of fine needle aspiration biopsy (FNAB) and CD3, CD20 and CD45 in the identification of oral primary diffuse large B-cell lymphoma (DLBCL).

Materials and methods: This is a descriptive clinical study. Ten patients clinically diagnosed as DLBCL in a tertiary level hospital in Qassim, Saudi Arabia; were included. Fine needle aspiration and incisional biopsies were taken from each patient and processed in the appropriate manner. Histopathological confirmation was done using haematoxylin and eosin stained specimens. Other sections were taken for the immune-histochemistry using a standard strept-avidin-biotin peroxidase procedure and monoclonal antibodies, including CD3, CD20 and CD45.

Results: The obtained results showed positivity of FNAB in 7 cases and three cases were inconclusive. Strong positive immune reaction was revealed with CD20 and CD45, while CD3 was negative in all studied cases.

Conclusion: These findings indicated the diagnostic significance of FNAB, CD20 CD45 and CD3 in proper diagnosis of oral DLBCL. More research is necessary for predicting the prognostic importance of these molecular markers in oral lymphomas.

Key words: B-cell lymphoma, CD3, CD20, CD45, FNAB.

أهمية استخدام خزعة الابر الدقيقة وبعض الدلالات الهستوكيميائية في التشخيص السليم لسرطان الغدد الليمفاوية الفموية الأولي من النوع-ب

الملخص

هدفت الدراسة الى استعراض أهمية استخدام خزعة الابر الدقيقة وبعض الدلالات الهستوكيميائية (سي دي-3 وسي دي-20 وسي دي-45)، في التشخيص السليم لسرطان الغدد الليمفاوية الأولي الفموي من النوع- ب.

تم اختيار 10 مرضى للمشاركة في الدراسة تم تشخيصهم اكلينيكيا بسرطان الغدد الليمفاوية الفموي الأولي من النوع- ب. تم أخذ خزعات جراحية وخزعات الابر الدقيقة من الفم وتم تجهيزها معمليا بالشكل المطلوب لإعداد الشرائح النسيجية اللازمة للفحص المجهري الداعم للتشخيص السليم، وكذلك الشرائح النسيجية المعالجة مناعيا لعمل فحص مجهري مناعي لكلا من سي دي-3 وسي دي-20 وسي دي-45.

خلصت الدراسة إلى أهمية استخدام خزعة الابر الدقيقة وبعض الدلالات الهستوكيميائية (سي دي-3 وسي دي-20 وسي دي-45) في التشخيص السليم لسرطان الغدد الليمفاوية الفموية الأولي من النوع- ب، مع أهمية عمل دراسات أخرى لرصد دور تلك الدلالات النسيجية التشخيصية في التنبؤ بالمرض.

الكلمات المفتاحية: تشخيص سرطان الغدد الليمفاوية الأولي من النوع-ب , سي دي 3 , سي دي 20 , سي دي 45 , خزعة الابر الدقيقة

Introduction:

Primary oral lymphomas are scarce and among different types diffuse large B-cell lymphoma (DLBCL) is the most widely recognized type. [1] DLBCL is prognostically typed as subgroups like germinal center B-cell like (GCB) and non-germinal center B-cell like (non-GCB) lymphomas based on gene and immunohistochemical expression .[2]

DLBCL is the highly prevalent subtype of non-Hodgkin's lymphoma (NHL) and is one among the five common malignant tumors, representing 30 to 40%. It occurs in bone marrow, gastrointestinal tract (GIT) and also in intraoral sites. [3] Though, the rate of oral primary DLBCL has not been previously listed in the literature.[1-4] The onset of DLBCL is usually occurs between 50-70 years of age with a slight male predilection than females.[5] In the oral cavity lesions usually occur in palate or maxillary vestibule as ulceroproliferative mass with symptom like pain, difficulty in eating.[6-8]

Diffuse proliferation of large neoplastic B cells with the same nuclear dimension of macrophage or larger than lymphocyte is the diagnostic criteria for DLBL. [9-11] It is a heterogeneous group of neoplasm characterized with inconstant genetic, immunophenotypic and clinical specifications that sometimes lead to misdiagnosis. Thus, the need for diagnostic parameters such as Fine needle aspiration biopsy (FNAB), CD45, CD3 and CD20 will be helpful in differentiation and in definite diagnosis of DLBCL from other variants. [12-14]

FNAB could play a vital role in the diagnostic workup for neoplastic and non-neoplastic oral lesions. It is non-expensive, less invasive, has more predictive value and provides rapid diagnosis and therefore could replace the open biopsy and avoid its potential complications.[15-16] Many scientific researchers have documented the efficacy of FNAB in establishing the correct diagnosis of lesions in the head and neck region.[17-23] FNAB is considered as an important tool in the initial diagnosis of Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL) in many studies, only few reports explored the prospective of FNAB for diagnosis of intraoral lesions, particularly oral lymphomas.[24-29]

CD3 is a co-receptor molecule. Protein tyrosine kinase (PTK) along with the T-cell receptors (TCRs) forms TCR/CD3 complex that plays a role in the signal transduction on antigenic stimulation.[30-35] CD3 molecules are located on 11q23 and 1q22 gene loci and are characteristically expressed on the surface of matured T lymphocytes. This could explain the possibility of using CD3 marker for differentiating between T and B lymphocytic lesions.[36-38] Many investigators described aberrant expression of specific T-cell antigen, CD3 in DLBCL in variable ages.[39-44]. CD20 or L26 is a transmembrane-embedded, non-glycosylated phosphoprotein of 33-37 KD, located on 11q12 gene locus and typically expressed on the surface of the precursors and matured B lymphocytes.[45] It plays a critical role in regulating the transmembrane conduction of Ca in

B lymphocytes and regulate its proliferation and differentiation.[46] Many studies reported the effective role of CD20 in identification of B-cell derived neoplasms.[47-52] Many similar studies documented the CD20 expression in B-cell lymphomas.[49-53]

CD45 or Leukocyte Common Antigen (LCA) is a monoclonal antibody that can be used to identify leukocyte cellular surface marker protein using immunohistochemistry in formalin-fixed paraffin-embedded human tissues.[54] In leukemia and NHL most of the neoplastic B and T lymphocytes will be stained and the neoplastic myeloid and erythroid cells are not stained. [55-59] Other reports showed that CD45 is a reliable marker for tissues in paraffin block fixed with formalin and can be a pan lymphocyte reliable marker for lymphomas.[30,31,54-59] The current research was done to assess the use of FNAB, CD3, CD20 and CD45 in proper diagnosis of oral primary DLBCL.

Materials and Methods:

The present descriptive research was carried out in the dental out patients department of King Fahd Specialist Hospital (KFSH) Al-Qassim, Saudi Arabia. Patients were screened for oral primary DLBCL from January 2016 to January 2017. Ten patients including both male and females were included and the fine needle aspiration was performed in the clinic of Histopathology/Cytopathology Unit of Prince Faisal Oncology Center, Buraidah, Al-Qassim, Saudi Arabia. The study was approved by the Institutional ethical committee. A written willingness was obtained from all participants included in this research.

Patient details including age, sex, site of involvement and clinical characters were obtained from the medical records. Povidone-iodine solution was used for preparation of the lesion site. The FNAB was done with 21–25 gauge needle with a 20-ml syringe without local anesthesia. The needle was inserted inside the lesion and aspirated after a pass, again the needle was inserted in a different direction and the procedure was repeated. The aspirated content was spread over a glass slides and 95% ethyl alcohol was used to fix for hematoxylin and Eosin (H &E) staining and air dried for Leishman staining. Proper diagnosis was made based on the cytological findings. An incisional biopsy was taken from each patient and all tissue specimens were fixed separately in 10% neutral formalin solution and then processed as paraffin wax blocks. Thin tissue sections of 5μm thick from the paraffin wax blocks are made and stained with haematoxylin and eosin (H & E) for histopathologic analysis and confirmation of diagnosis in all cases. Cytopathological diagnosis was correlated with the histopathological diagnosis.

Other sections 4μm thick were taken from the specimen blocks and processed for immunohistochemistry using a standard strept-avidin-biotin peroxidase procedure,[50] and monoclonal antibodies including CD3, CD20 and CD45 at a dilution of 1:100. The chromogen substrate for development of the peroxidase activity was 3, 3' Diaminoben-

zidine (DAB). The universal kit, the monoclonal antibodies and the chromogen were purchased from DAKO, glostrup, Denmark. Tissue sections were counterstained with Mayer's haematoxlyin, dehydrated and cover slipped with a permanent mounting medium. To evaluate the immune-histochemical staining grades, at least 10 high power fields throughout the tumor population exhibited cytoplasmic staining sections were examined in each case. Immunoreactivity by more than 10% of the tumor was considered positive and if 10-25% of tumor cell get stained it is considered as weak positive staining, 26-50% is mild, 51-75% is moderate and 76-100% is strong. Statistical analysis of the obtained data in all studied cases was performed by using the Chi-square test to evaluate the predictive or significant value of FNAB, CD3, CD20 and CD45 in proper diagnosis of oral primary DLBCL.

Results:

Clinical Characteristics:

Among the participants six patients were males and four were females. Patients were ranging in age from 45 - 65 years. All studied cases showed clinically diagnosed tumors of different intraoral sites, including floor of the mouth (2/10 cases), posterior tongue (2/10 cases), soft palate (2/10 cases), retro molar area (2/10 cases), buccal mucosa (1/10 case) and maxillary vestibule (1/10 case). Intra-oral examination of nearly all involved sites showed slowly growing painless soft tissue mass of variable size without ulceration or other surface changes. Participants had no signs of other diseases in the body.

Fine Needle Aspiration Biopsy:

Ten cases of intraoral DLBCL were evaluated by FNAB before histopathological examination. Seven (70%) cases were cytologically diagnosed as DLBCL and three cases (30%) were inconclusive or atypical, but all were found to be true DLBCL on histological examination (Figure 1).

Histopathological Examinations:

Evaluation of H&E stained slides from each specimen was independently carried out. Agreement was reached in all 10 cases, showing classic morphologic features of DLBCL. Sections showed neoplastic lymphoid tissue containing sheets of diffuse large, round or oval shaped atypical lymphoid cells with big round nuclei, prominent nucleoli with basophilic cytoplasm (Figure2). A few immunoblasts and scattered mature lymphocytes were also seen throughout the lesion.

Comparative analysis of FNAB with Histopathologic Features:

It was interesting to note that all of DLBCL lesions reported in FNAB matched with the histopathological findings of DLBCL. While three inconclusive cases with FNAB also showed the histopathological features of true DLBCL.

Immunohistochemical Findings:

Immunohistochemical results of all tissue samples of this study are summarized in table1. Immunoreactivity was independently assessed by the investigator to reach agreement on all examined cases. The immunohistochemical expression of CD3 monoclonal

antibody was negative in all 10 specimens (Figure 3). On the other hand, immunostaining of CD20 immune marker revealed strong positive cytoplasmic membrane staining of tumor cells in all studied cases (Figure 4). CD45 expression was obviously evident in all cases of this study as indicated by presence of strong positive cytoplasmic membrane localization of the neoplastic cells (Figure 5). CD3, CD20 and CD45 immune markers were significantly associated in all studied cases (Figure 6).

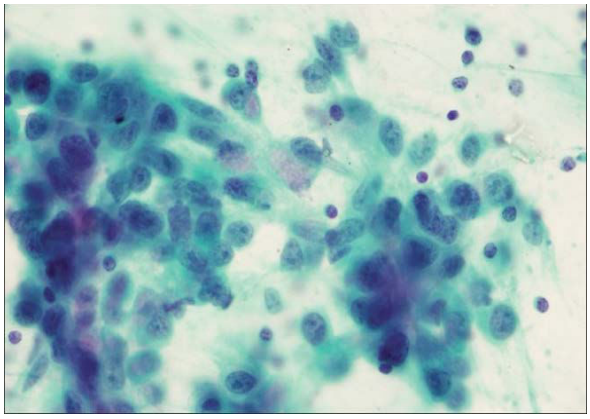


Figure (1): FNAB showing large, round and oval shaped atypical lymphoid cells with large round nuclei, prominent nucleoli and basophilic cytoplasm. (Leishman stain, X 200)

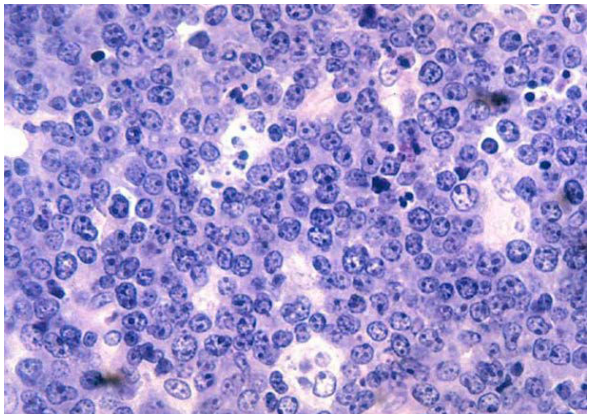


Figure (2): DLBCL showing diffuse large, round and oval shaped atypical lymphoid cells with large vesicular round nuclei, prominent nucleoli and basophilic cytoplasm with few immunoblots scattered throughout the lesion. (H&E stain, X 200)

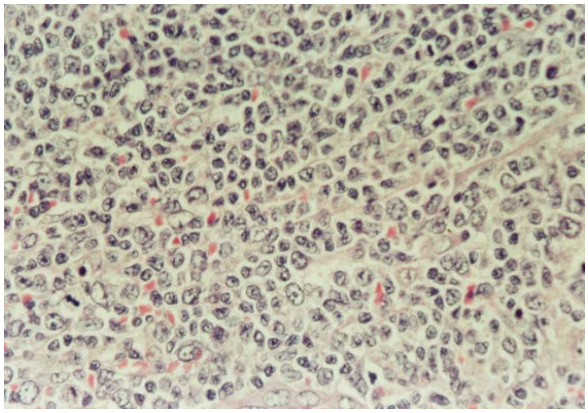


Figure (3): Immunohistochemical expression of CD3 showing negative staining of the abnormal lymphoid cells (strept- -avidin -biotin tech., X200

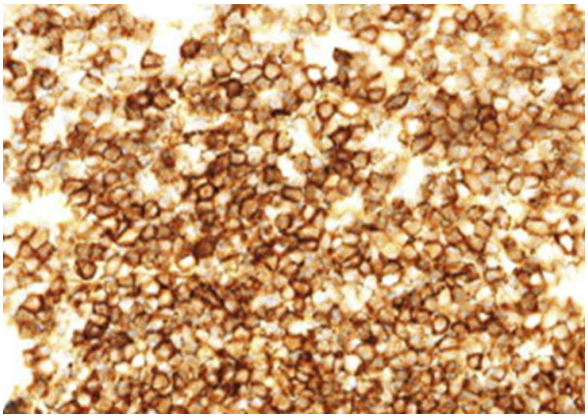
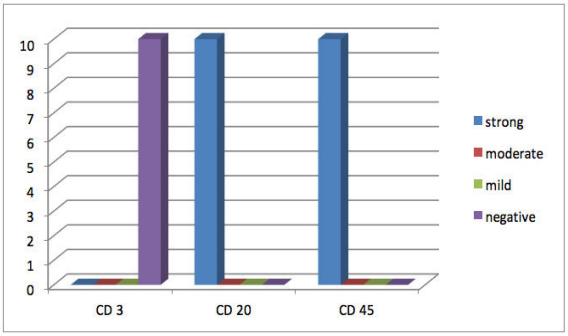


Figure (4): Immunohistochemical staining of CD20 showing strong positive staining of atypical lymphoid cells (strept- -avidin -biotin tech., X200)



Figure (5): Immunohistochemical staining of CD 45 showing strong positive cytoplasmic reaction of atypical lymphoid cells (strept- -avidin -biotin tech., X200).

Figure (6): Diagram showing immunohistochemical expression of CD3, CD20 and CD45 immune markers in oral DLBCL



Discussion:

Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) are the two types of lymphomas, the malignant neoplasm arising from lymphoid tissue. [53] DLBCL is one of the most common subtypes of NHL. The biology of DLBCL has been clarified greatly by studying their morphologic findings, antigenic features and immunologic properties. New reagents (such as monoclonal antibodies), new techniques (such as immunohistochemistry) and new instruments have significantly enhanced the understanding of DLBCL.[60,61] Correlation of clinical and immunologic findings is crucial at arriving at correct diagnosis of not only DLBCL, but also in all lymphomas. [62-63]

The current research depicts the significant role of FNAB and some monoclonal antibodies, including CD3, CD20 and CD45 in definite diagnosis of oral DLBCL. FNAC/FNAB is a relatively less-invasive and valuable procedure that provides helpful information about neoplastic and non-neoplastic intraoral and oropharyngeal lesions. In the current study ten cases of intraoral DLBCL were evaluated by FNAB before histopathological and immune-histochemical examinations. Seven cases (70%) were diagnosed as DLBCL and 3 cases (30%) were inconclusive or atypical, but were found to be DLBCL on histological examination. This finding indicated that the precision of the FNAB method in proper diagnosis of DLBCL. According to Florentine et., al FNAB results have accuracy similar to those of the regular biopsy.[64] Similar studies described the importance of FNAB in establishing the diagnosis of oral and oropharyngeal lesions with high grades of sensitivity, specificity and accuracy.[17-29]

The immunohistochemical expression of anti-CD3, a protein tyrosine kinases (PTK) with the T-lymphocytes receptors (TCRs) forming TCR/CD3 unit that is concerned with the signal transmission on antigenic stimulation, in all 10 DLBCL cases studied was negative. Our results agreed with the findings of many investigators, who demonstrated the negative immunoreactivity of anti-CD3 in neoplasms of T-cell types. [65-70] However, such CD3 specificity was also confirmed in the third and fourth international workshops and conferences on human leucocytes differentiation antigens. [71-73]

It has been widely accepted that advances in cellular immunology have provided insight into the normal pathways of lymphocytes development and the cellular origin of lymphoid neoplasms. Also, with the development of monoclonal antibodies directed

against cell-surface antigens that distinguish B and T lymphocytes at defined differentiation stages, it become apparent that the immunohistochemical expression of CD20, a trans-membrane embedded, non-glycosylated phosphoprotein of 33 to 37 KD, expressed throughout the B-cell life, was strongly positive in all studied cases of oral DLBCL. [74] This finding explains the possibility of uses of CD20 as a reliable marker for distinguishing B-cell lymphoproliferative diseases from T-cell diseases. Consistent with this context, many previous studies reported the positive expression of CD20 in virtually all cases of B-cell disorders. Thus, CD20 expression is of greater diagnostic value than other markers for distinguishing tumors of B-cell origin as oral DLBCL. [74-78]

Finally, Immunohistochemical expression of CD45 / LCA in all intraoral DLBCL cases of this study demonstrated strong positive cytoplasmic immunoreactivity of the neoplastic lymphoid cells. This finding was in agreement with that of many other studies performed on DLBCL of non-intraoral locations.[54-59] Thus, it is generally accepted that the expression of LCA in neoplasms of lymph nodes and extra nodal sites of the oral cavity is an excellent indicator of cellular origin and it should be used for this purpose.

In conclusion, the current research showed the importance of FNAB and immunohistochemical identification of CD3, CD20 and CD45 for a specific diagnosis of oral DLBCL. Although the clinical and therapeutic significance of their expression in DLBCL is still not fully understood, further research is necessary to confirm the prognostic importance of these molecular markers particularly in oral lymphomas.

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