Detection of Cancer Stem Cells in the Most Common Head and Neck Non-Hodgkin's Lymphoma Using CD34: in Vitro Study

Hadya N. Ghedan[1],Samia M. El-Azab[2], Safa F.Abd El-Ghany[3], Mona A.Saker[4]

[1]B.D.S, Faculty of Oral and Dental Medicine, Cairo University, Egypt
[2]Oral and maxillofacial pathology, Faculty of Oral and Dental Medicine,Cairo University, Egypt
[3]Oral &Maxillofacial Pathology, Faculty of Oral and Dental Medicine, Cairo University, Egypt
[4]Pathologydepartment, National Cancer Institute, Cairo University, Egypt
Corresponding Author: Hadya N. Ghedan

Abstract:
Background.Non-Hodgkin Lymphomas(NHL) are a heterogeneous group of malignancies of the immune system;diffuse large B-cell lymphoma and Burkitt's lymphoma are among its most common subtypes. Recently, it is widely believed that cancer stem cells play critical roles in the initiation, metastasis, and relapse of cancers. However, no sufficient data supported the presence of cancer stem cells in NHL of the head and neck.
Purpose. To detect cancer stem cellsin diffuse large B-cell lymphoma and Burkitt's lymphoma using immunohistochemical expression CD34, a widely used marker for identification and detection of hematopoietic stem cells.
Methodology.The present work was performed on archival paraffin blocks of diffuse large B-cell lymphoma and Burkitt's lymphoma. The mean count and mean area of the positive CD34 cells were assessed by the image analyzer computer system using the software Leica Qwin 500.
Results.The results showed positive CD34 immunoreaction in 90% of the diffuse large B-cell lymphoma cases, and 70%of the Burkitt's lymphoma cases, denoting presence of cancer stem cells in both tumors. Diffuse large B-cell lymphoma showed significantly higher mean count and mean area of positive CD34 cells than those of Burkitt's lymphoma.
Conclusion.Cancer stem cells are present in both diffuse large B-cell lymphoma and Burkitt's lymphoma and may be a common origin of these lesions. Diffuse large B-cell lymphoma may be more aggressive than Burkitt's lymphoma. The level of cancer stem cells may be a valuable prognostic tool which is directly proportional with more aggressive behavior and poor prognosis.CD34 is a valuable cancer stem cell marker in diffuse large B-cell lymphoma and Burkitt's lymphomaand can be used as prognostic marker in these lesions.
Key words: CD34,Non-Hodgkin's Lymphoma (B-Cell lymphoma and Burkitt's lymphoma),Cancer Stem Cells

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I. Introduction

Non-HodgkinLymphomas (NHLs)are heterogeneous group of lymphoproliferative disorders originating from B-lymphocytes, T-lymphocytes or Natural Killer (NK) lymphocytes.[1, 2]Diffuse large B-cell lymphoma (DLBCL)has been reported as the most common subtype of aggressive B-cell lymphoma worldwide, which accounted for 25–30% of adult NHL and showed considerable biologic and clinical heterogeneity.In Egypt, it has also been reported as the most common subtype constituting 49% of cases as reported in the National Cancer Institute (NCI) Cancer Pathology Registry.[3,4]

On the other hand, Burkitt's lymphoma (BL) was first described by Dermis Burkitt (1958) in African children with large jaw in relation to Epstein-Barr virus. It is a highly aggressive B-cell neoplasm which has been characterized by the translocation and deregulation of the c-myc gene on chromosome 8. It has been reported as a rapidly growing neoplasm requiring immediate diagnosis and treatment.[5, 6]

Cancer stem cells (CSCs), also known as tumor-initiating cells, or tumor propagating cells are cancer cells that possess capability of proliferation, differentiation, and self-renewal. It was widely believed that CSCs played critical roles in the initiation, metastasis, and relapse of cancers.[7,8]Moreover; they may have possible roles in cancer therapy. However, specific recognition of these cells fromtumour masseshas represented the first challenge.CSCs have been detected and isolated using various methods, including overexpression of specific cell surface markers. Among these surface markers, CD34 has been widely used as a marker for identification and isolation of hematopoietic stem cells (HSCs).[9,10,11]It is a 105-120-kDa transmembrane glycoprotein which was first identified in 1984 on hematopoietic stem and progenitor cells.[12,13]

To date, no sufficient data supports the presence of CSCs in lymphoma, although they were detected in many cancers.[14, 15]Since, the lymphoma stem cell hypothesis has remained largely unexplored, it seems interesting to detect the presence of CSCs in lymphoma. This could contribute to better understanding of the role of CSCs in development, tumorgenesis, prognosis and treatment of oral NHL.

II. Material And Methods

2.1. Tissue specimens

A total of 30 specimens of NHL from cervical lymph nodes were retrieved from the archival paraffin blocks from files of Pathology Department, NCI, Cairo University. They comprised DLBCL (20 cases) and BL(10 cases).

2.2. Histopathological and IHC staining

All the procedures took place at pathology lab, NCI, Cairo University.

Thin (4 microns) paraffin sections of each tissue specimen were stained with Haemotoxylin and Eosin (H&E) to reconfirm the diagnosis and the most representative sections were selected. Paraffin sections were mounted on positively charged glass slides (Optiplus, Biogenex, USA) for immunostaining.

The initial immunohistochemical panel for lymphomas [CD-3, CD-20, CD10 and terminal deoxynucleotidyltransferase (TDT) antibodies, Ventana Medical Systems, USA] was used to confirm the diagnosis. CD-3 (T-cell marker, rabbit monoclonal antibody, catalogue#790-4341) and CD20 (B- cell marker, mouse monoclonal antibody, catalogue#760-2531) were used for both DLBCL and BL, to confirm the B cell origin and exclude the T-cell one. Furthermore, CD-10 (rabbit monoclonal antibody, catalogue#790-4506) and TDT (rabbit polyclonal antibody, catalogue#760-2670) were used on the BL cases to identify whether the B cells were mature or immature. Staining for each antibody was reported as positive or negative immunoreaction.

Then, the selected cases were stained by anti-CD34 mouse monoclonal antibody (catalogue#790-2927) and iVIEW detection kit(catalogue#760-091) (Ventana Medical Systems, USA), using the Bench Mark XT system (Ventana autostainer, USA).

2.3. Assessment of immunostainig

All the cells that showed positive CD34 immunoreactivity were considered as CSCs. They were detected by brownish color in the cytoplasm and cell membrane. Immunostainig of CD34 was assessed inResearch Unit of Oral and Maxillofacial Pathology Department, Faculty of Oral and Dental Medicine, Cairo University. Image analyzer computer system was used for counting of CD34 positive cells and measuring area of positive CD34 immunoreaction of tumor cells.

2.4. Counting of CD34 positive cells

The number of CD34 positive cells on magnification (X400) was counted in 5 randomly chosen fields using manual counter across the entire sample by two investigators.

2.4.1 Measuring area of positive reaction of tumor cells

Areas of positive CD34 immunoexpression in tumour cells were chosen for evaluation, and the positive reaction in blood vessels was excluded.

2.4.2. Statistical analysis

The data obtained from the image analyzer computer system were tabulated and statistically analyzed. Data was presented as mean± standard deviation (SD) values. Student's t test was used for comparing mean count and meanarea of positive CD34 immunoreaction between both DLBCL and BL. The P values were considered as the following: not significant (P value > 0.05),significant (P value ≤ 0.05),or highly significant (P value ≤ 0.01).

III. Results

3.1. Immunohistochemical findings

All the examined cases of DLBCL and BL (100%) showed positive CD20 immunoexpression and negative CD3 immunoreaction, denoting their B cell origin. The BL cases (100%) showed positive CD10 and negative TDT immunoreaction, denoting that the involved B-cells were mature.

Positive CD34 immunoreactivity was detected in 18 out of 20 examined DLBCL cases (90%), and 70% of BL cases (7/10). Cytoplasmic and membranous CD34 immunoexpression was restricted to CSCs and endothelial cells. The CSCs were either sporadic or arranged in small groups (Fig. 1 and 2).
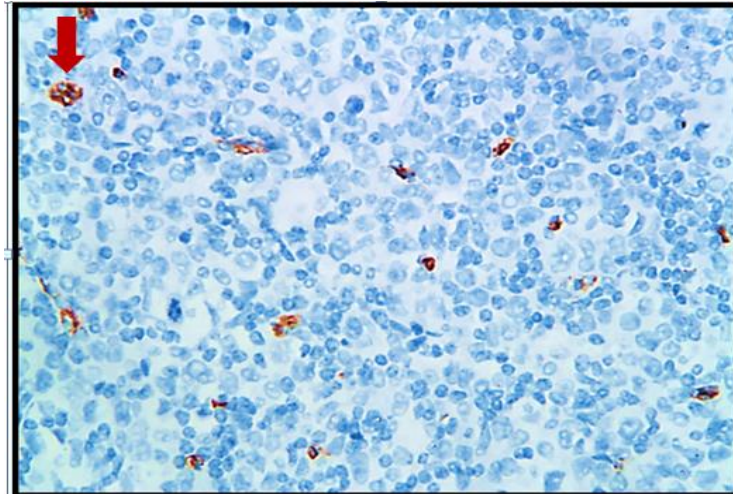
Fig. 1. A photomicrograph of DLBCL showing strong membranous immunoreaction of CD34 in CSCs, which are either sporadic or in small groups (arrow), anti-CD34
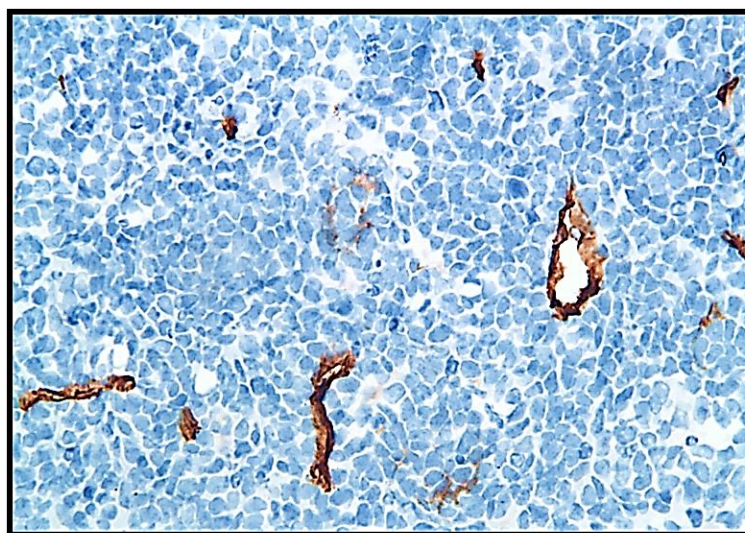
Fig. 2.A photomicrograph of BL showing strong immunoreaction in blood vessels lined by endothelial cells, anti-CD34 antibody, ×400.

3.2. *Statistical analysis*
Both the mean count and the mean area of CD34 immunoreactivity were higher in DLBCL than BL, which reflects the increased number of CSCs in DLBCL than BL (Table 1) (Fig. 3 and 4).

Table 1. The meancount & mean area of CSCs in both DLBCL and BL Groups.

	Group	Mean ±SD	P value
Mean count	DLBCL	10.90±2.77	0.0119[*]
	BL	7.30±2.98	
Mean area	DLBCL	269.29±81.34	0.0087[**]
	BL	167.28±73.61	

[*] $P < 0.05$ Significant
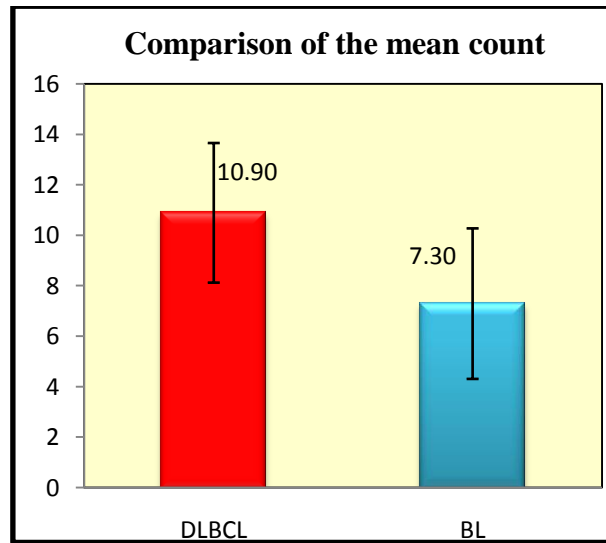[**] $P < 0.01$ highly Significant

Fig (3): Bar chart showing the **mean count** of CSCs in both DLBCL and BL groups.
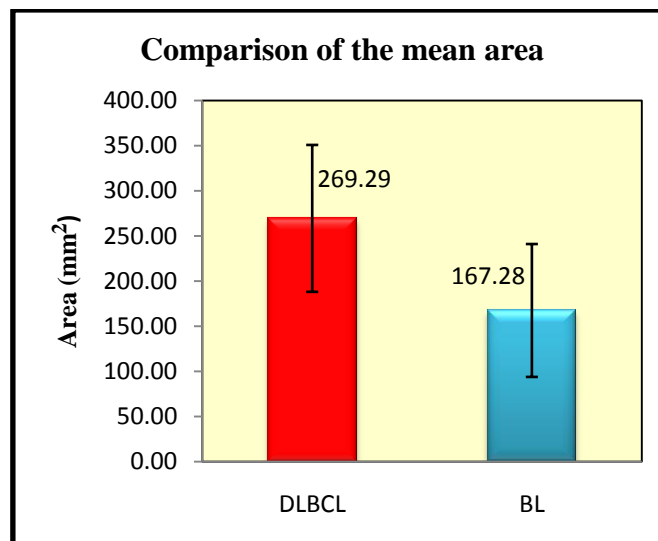
Fig (4): Bar chart showing **mean area** of CSCs in both DLBCL and BLgroups.

IV. Discussion

Two models were proposed to explain tumor development: the conventional "stochastic model" and the "CSC model". The former propounds that every cancer cell has the ability to be the founder of a new tumor. Recently, the "CSC model" has proposed that cancer evolution occurs from tissue progenitor or stem cells which have a "deregulated" self-renewal pathway. Several studies confirmed the potential role of CSCs in the pathogenesis of cancers. However, no sufficient data supported the presence of CSCs in NHL. [16,17]

Various methods have been used for detection and isolation of CSCs; howevercell surface marker detection has shown a major advantage of obtaining a precise population compared to other methods.In the present study, CD34 immunostaining was precisely localized to a small population of cells which were considered as CSCs. This was in agreement with what has been reported by Sassetti et al. (2000) and Pisacane et al. (2007), who pointed out that expression of CD34 was maintained in the undifferentiated phenotype of progenitor cells and was not present on mature hematopoietic cells. [18,19]

In the present study, CSCs were detected successfully in the most common subtypes of NHL. These cells could play an important role in the development, tumorgenesis and predication of prognosis of these lesions. Moreover, both DLBCL and BL might share a common origin which is CSCs derived from HSCs that are responsible for tumor growth and formation of both CSCs as well as the non tumorgenic progeny that form bulk of tumor mass. [16]

Presence of CSCs is in agreement with the results of Bonnet and Dick (1997), Cozzio et al. (2003) andJanikova et al. (2009)whoconfirmed presence of CSCs in hematopoietic malignancies. [20,21,22] Also,Nakashima et al. (2010), Shafer et al. (2010) andIkeda et al. (2010)pointed out that HL had CSCs. [23,24,25]

Similarly,Vega et al. (2010)andJung et al. (2011)supported the existence of lymphoma SCs in studied mantle cell lymphoma.[26,27] More recently, Kim (2011) and Lee et al. (2013)supported that B-cell NHL could be enriched with lymphoma SCs.[28,29]

In the same context, CSCs have now been isolated from several human malignancies, as lung tumors (Gibbs et al., 2005), bone sarcomas (Gutova et al., 2007)and pancreatic carcinomas (Hermann et al., 2007).[30,31,32] In contradiction to our results, Kim et al. (2013) failed to detect CSCs in canine lymphoma.[33]

The findings of the present study also shed the light on the prognostic value of CSCs as a sign of more aggressive behavior and poor prognosis. That's why we propose that DLBCL could have a more aggressive behavior than BL as it showed higher mean count and mean area for CD34 positive cells, denoting an increased number of CSCs. This could explain the different invasion potentials of these lesions and recommending different treatment modalities for each of them(Diaz-Cano, 2012).[34]

Also, Kim (2011) and Lee et al. (2013)reported the association of CSCs with malignant potential of B-cell lymphomas.[28,29] In the same context,Zhang et al. (2008),Beier et al. (2008), Jiang et al. (2009),Su et al. (2010),Liu et al. (2015)and Su et al. (2015)agreed thathigherCSC levels and high expression of CSC markers were associated with advanced disease stage, high histologic grade, and poor clinical outcome.[35, 36,37,38,39,40]

On the contrary, some studies proposed that the increase in CSCs had a favorable effect on tumor behavior as Costa et al. (2012) who reported that low expression of CSC markers was associated with higher probability of disease progression and death.[41] Similarly, Kasem et al. (2012)found that solid pattern of adenoid cystic carcinoma was associated with low expression of CSCs marker.[42]

V. Conclusions

CSCs are present in both DLBCL and BL and may be a common origin of these lesions. DLBCL may be more aggressive than BL. The level of CSCs may be a valuable prognostic tool which is directly proportional with more aggressive behavior and poor prognosis.CD34 is a valuable CSC marker in DLBCL and BL andcan be used as prognostic marker in these lesions.

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