CASE REPORT

BURKITT LYMPHOMA WITH ABERRANT IMMUNOPHENOTYPE IMPOSING DIAGNOSTIC CHALLENGE

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Background: Burkitt lymphoma (BL) exhibits a characteristic immunophenotype that is positive for pan–B-cell antigens and germinal center markers while negative for immature markers. A deviation from classic immunophenotype can cause diagnostic confusion and might result in false exclusion of BL. In some cases, overlapping clinical, morphological and immunophenotypic features of BL and B lymphoblastic lymphoma (B-LL) can be of diagnostic challenge. However, definitive delineation is of paramount importance due to difference in treatment. We describe a case of BL in a child with atypical features including absence of L3 morphology in diagnostic tissue and aberrant expression of CD34, CD99 and BCL2 on immunohistochemistry. These findings led to the interpretation of B-LL which was later on excluded by detection of t (8;14). This unorthodox case not only highlights the importance of cytogenetic testing but also emphasizes the correlation of all the diagnostic tools before making a definitive diagnosis. Therefore, reporting this case will help in eliciting the high index of suspicion among pathologists for this exceptionally unusual immunophenotype.

Keywords: Burkitt lymphoma; Aberrant immunophenotype; Cytogenetic; Paediatric

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INTRODUCTION

Burkitt lymphoma (BL) is an aggressive B-cell Non-Hodgkin lymphoma that affects both children and adults. The overall incidence is 2–2.5% in adults however it accounts 30–50% of all childhood lymphomas.[1,2] A regional study from Pakistan in a tertiary care hospital reported that BL is the most common histopathologic subtype of lymphomas that is seen in our paediatric population.[3]

Three distinct variants of BL are recognized (endemic, sporadic and immunodeficiency associated) with different geographical distribution and clinical presentations. Childhood sporadic BL is a distinct entity which has classic morphology and immunophenotype. In these cases, the cells are monotonous, medium sized and exhibit high nuclear to cytoplasmic ratio, round nuclei and single to multiple prominent nucleoli. Cytoplasm is moderate to abundant, deeply basophilic and show prominent vacuoles. High mitotic rate is a characteristic feature of BL, with a "starry sky" pattern that results from numerous intermixed tangible body macrophages phagocytizing apoptotic debris. All cases of BL are of B lineage, shows strong positivity for B cell markers while immature markers are typically negative. The proliferative index (Ki-67) is nearly 100% (at least 95% of tumour cells), due to the short doubling time of tumour cells. The molecular hallmark of Burkitt lymphoma is the translocation of MYC at band 8q24 to the IGH region on chromosome 14q32, t(8;14)(q24;q32) or less commonly to the IGK locus on 2p12 t(2;8) or the IGL locus on 22q11 t(8;22). The classic translocation occurs in up to 80% of cases while variant occurs in the remainder. [1]

We present a case of BL with atypical morphologic features in abdominal mass biopsy and aberrant immunophenotype which led to an initial impression of B-LL. Later on, the clinical behaviour of patient and bone marrow findings raised suspicion of BL; hence cytogenetic analysis for c-MYC was done that revealed t(8;14).

Knowledge and recognition of these cases can help to improve awareness regarding consideration of cytogenetic workup irrespective of clinical presentation, morphology and immunophenotypic findings. The main purpose of this case report is to elicit the sensitivity that pathologists must consider and correlate all the diagnostic tools including cytogenetic before signing out such ambiguous cases.

CASE REPORT

A 6-year-old male child presented with fever, weight loss and abdominal distention for the last two months. On examination there was cervical lymphadenopathy, hepatomegaly and mass in epigastrium. CBC showed haemoglobin 10.7g/dl, reactive neutrophil leucocytosis and thrombocytosis. No abnormal/atypical cells observed on blood film examination. CT abdomen showed large heterogeneous predominantly hypoechoic lymph nodal mass in epigastrium extending into the pelvis along with hypo echoic lesion in liver.

Ultrasound guided tru-cut biopsy of abdominal mass showed a neoplasm exhibiting nesting pattern with focally diffuse areas (Figure-1). The tumour revealed medium to large sized cells with scant cytoplasm and

nuclei with irregular nuclear membranes, pleomorphism and hyperchromasia along with occasional prominent nucleoli and fine nuclear chromatin. Immunohistochemical studies showed diffuse membrane positivity for CD79a, CD20 and CD10. Among immature markers, Tdt was negative, whereas CD34 showed patchy positivity in malignant cells. CD99 and weak BCL2 expression was also noted. CD31 was negative in tumour cells and highlighted blood vessels. Ki-67 labelling index was approximately 95%. Based on this morphology and immunophenotype, the case was diagnosed as B-Lymphoblastic Lymphoma. Bilateral bone marrow biopsy was done as a part of staging workup. Bone marrow aspirate showed infiltration by medium sized mononuclear cells characterized by high nuclear to cytoplasmic ratio, granular nuclear chromatin and scant to moderate amount of basophilic cytoplasm. Some cells showed punched-out cytoplasmic vacuolations (Figure-2). Trephine biopsy also revealed interstitial infiltration by mononuclear cells admixed with normal hematopoietic precursors. Immunophenotyping showed bright positivity for B cell markers including CD79a and CD20. However, Tdt and CD34, the immature markers, were found to be negative. The absence of immature markers along with L3morphology on bone marrow raised the question of diagnosis.

Therefore, cytogenetic studies including karyotype and Fluorescence in situ hybridization (FISH) for BCR/ABL1, ETV6/RUNX1, MLL gene rearrangement, and t(8;14)(q24; q32) were performed. Karyotype showed normal 46,XY pattern, however, t (8;14) was detected by FISH (Figure 3). Based on this finding, diagnosis was revisited, and an addendum report of BL was issued from histopathology as well as haematology sections and patient shifted to NHL treatment protocol. Chemotherapy was well tolerated and currently patient is in complete remission (CR).

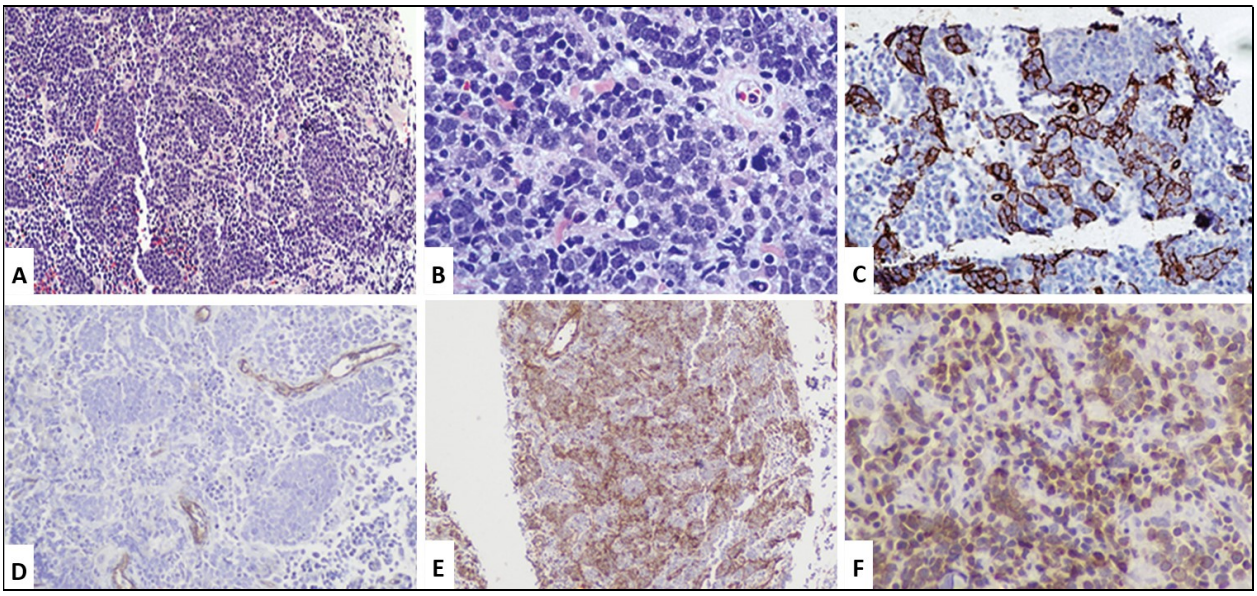

Figure-1: (A) Low power view of H & E stained section of abdominal mass biopsy showing nesting pattern of malignant cells (B) High power view of H & E stained section exhibiting medium sized cells with irregular nuclear contours and pleomorphism (C) CD 34 showing bright positivity in aggregates of malignant cells (D) CD31 positive in blood vessels, negative in malignant cells (E) CD99 positive (F) BCL2 variable positive.

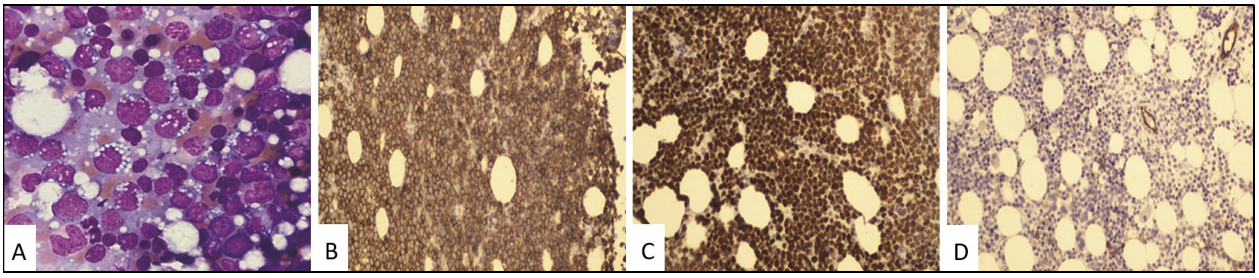

Figure-2: (A) Geimsa stained bone marrow aspirate exhibiting medium to large sized cell with high nuclear to cytoplasmic ratio, basophilic cytoplasm and punched out vacuoles (B) CD20 show diffuse bright positivity (C) Ki-67 Approximately 95-99% (D) CD34 Negative

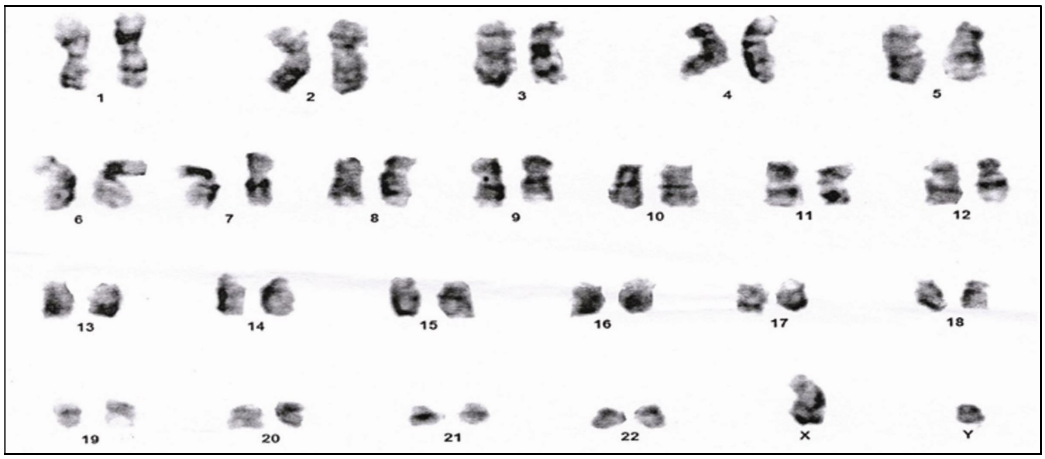

Figure-3: Karyotype 46, XY

DISCUSSION

Distinction between BL and B-LL is of profound clinical importance due to difference in disease biology, treatment protocol and duration, and prognosis. BL is an aggressive yet highly chemo-sensitive malignancy with a reported cure rate of 80–90% on recent treatment regimens.[4] It presents with heterogeneous clinical features however classical morphology and typical immunophenotype usually helps in diagnosis.

Virtually all cases of BL show L3 morphology and strong positivity for B cell markers including CD20, CD79a, CD22, CD19 and PAX 5 in addition to germinal center markers bcl-6 and CD10. BL cases are characteristically negative for CD5, CD23, BCL2 and immature markers Tdt, CD34 and CD99.[1] However, in the current case, morphology was favouring lymphoblastic lymphoma on diagnostic tissue (abdominal mass) biopsy along with expression of CD34, CD99, and BCL2 on immunohistochemistry. CD34 is a well-known marker of immaturity, generally seen in myeloid and lymphoblastic leukaemia whereas its expression in BL is exceptionally rare.[5] A recently published study by Demina *et al* showed significant difference (*p*-value <0.0001) between BL and B-LL with none of the case of BL showed CD34 expression. The most recent version of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues stated that approximately 2% of otherwise classic paediatric BL cases have a phenotype of precursor B cells, i.e., Tdt and sometimes CD34 expression.[1,5]

Expression of BCL2 and CD99 were other unusual immunohistochemical findings in our case. Aberrant and weak expression of BCL2 in variable number of cells is reported in 10–20% of cases only.[6]

High BCL2 expression in BL suggests presence of an additional BCL2 breakpoint which is consistent with high grade lymphoma with MYC and BCL2 and/or BCL6 rearrangements. Neus et al conducted a study on clinical and pathological features of BL expressing BCL2; they analysed 150 cases of BL and found positivity of variable intensity in 23% of cases. However, no significant difference observed in terms of clinical presentation and outcome of BCL2 positive and BCL2 negative subgroups of BL cases. Similarly, positivity of CD99 is reported in few cases of BL but it is characteristically observed in precursor B- and T-cell lymphoblastic lymphomas/leukemias. A study on immunoreactivity of CD99 was conducted by Sung et all and they reported its expression in only 2 out of 18 (11%) of the BL cases. Hence the co-expression of CD34, CD99 and BCL2 in absence of classic BL morphology imposed a diagnostic challenge in our case and resulted in a misdiagnosis of B-LL.

Further, in staging workup, bilateral bone marrow biopsy was performed which in contrast to the findings of abdominal mass biopsy, revealed infiltration by medium sized cells with morphological and immunohistochemical features suggestive of BL. Due to these discrepant findings, cytogenetic testing by FISH was requested on trephine tissue for t(8;14) and found to be positive. Although this translocation is considered as diagnostic feature of BL, Kimiyoshi *et al* recently studied cases of B-cell precursor immunophenotype and reported that 8q24/MYC rearrangement (8q24/MYC-r) is also occasionally observed in B-LL.[7,8] Previously published studies also showed that BL with MYC-r that display B cell precursor immunophenotype has some resemblance with B-LL in terms of disease biology.[9,10]

Despite of similarities in these two entities, BL patients with precursor immunophenotype showed inferior outcome when treated with ALL protocols however B-LL patients with t(8q24)/MYC-r responded well to BL type

chemotherapy.[9] Based on clinical findings, morphological and immunohistochemical features of bone trephine and lymph node biopsy, and cytogenetic studies we classified this case as Burkitt lymphoma with aberrant immunophenotype. Hence patient was shifted to BL type protocol as better outcome with this strategy is evidenced in literature.[4,10]

CONCLUSION

This case, a B-lineage malignancy with immature immunophenotype with high proliferation index and t(8;14) remains a diagnostic dilemma. Though BL and B-LL both are B-cell lymphoproliferative disorders but precise discrimination is crucial for selection of appropriate treatment protocol. Morphology and immunophenotyping is the mainstay of diagnosis in BL, however in challenging cases with atypical findings such as this one requires comprehensive profiling that includes cytogenetic studies at an initial phase of workup to avoid delay/misdiagnosis.

Declarations:

Ethics approval: Not applicable

Consent for publication: Informed consent was taken from the parents of patient.

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