INTRODUCTION

Non-Hodgkin lymphoma (NHL) is the fourth most common childhood malignant tumors in the United States,[1] Burkitt lymphoma (BL) accounts for 30–50% of all pediatric lymphomas. The aim of this study was to investigate the clinicopathologic features, immunophenotype, Epstein-Barr virus (EBV) infection and c-myc gene rearrangement of sporadic BL in children.

METHODS

Case selection

Ninety-two cases of pediatric BL were retrospectively analyzed for clinical features, immunohistochemistry, EBV-encoded RNA (EBER) status by in situ hybridization and c-myc gene rearrangement by fluorescence in situ hybridization.

Results:

In the 92 cases, male is predominant in sex distribution (M: F = 3.38:1). The average age at diagnosis was 4.97 years. Polypoid BL showed a lower clinical stage ($P = 0.002$), and advanced clinical stage and low serum albumin level at diagnosis were associated with poor outcome ($P = 0.024$ and 0.053, respectively). The positive expression of CD10, B-cell lymphoma-6, MUM1 and EBER were 95.7% (88 cases), 92.4% (85 cases), 22.8% (21 cases), 41.3% (38 cases), respectively. The expression of MUM1 were not associated with EBV infection status ($P = 1.000$). c-myc gene rearrangement was detected in 94.6% (87/92). Clinical treatment information for 54 cases was collected, 21 patients died of tumor after surgery alone, 33 patients received surgery and chemotherapy, and of which six patients died shortly afterwards (MUM1 positive expression in 3 cases, $P = 0.076$).

Conclusions:

The anatomical location, growth pattern and serum albumin level of BL were associated with biological behavior. MUM1 may be a potential adverse prognostic marker, and not associated with EBV infection status.

Key words: Burkitt Lymphoma; Epstein-Barr Virus; Interphase Fluorescence In Situ Hybridization; MUM1 Protein

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is the fourth most common childhood malignant tumors in the United States,[1] Burkitt lymphoma (BL) accounts for 30–50% of all childhood lymphomas, is defined by the World Health Organization (WHO) classification 2008 as a B-cell lymphoma (BCL) with an extremely short doubling time that often presents in extra nodal sites or as an acute leukemia.[2] BL is a highly aggressive NHL and characterized by c-myc gene translocation. Three clinical variants of BL are recognized, including endemic BL, sporadic BL and immunodeficiency-associated BL. Sporadic BL is widespread, mainly in children and adolescents.[2] In order to understand the clinicopathology, immunophenotype, c-myc gene rearrangement and Epstein-Barr virus (EBV) infection of BL, and investigate the relationship between clinical parameter and prognosis, 92 cases of BL of children were retrospectively study for the clinical and pathological features.

METHODS

Case selection

Formalin-fixed and paraffin-embedded biopsies from 92 children (all younger than 14 years old) diagnosed with BL between 1984 and 2013 were retrieved from the Pathology Department of Jiangxi Children’s Hospital and the Pathology Department of Jiangxi Province Tumor Hospital. The pathological diagnosis was reevaluated by two chief physician (W.Y., C.H) according to the 2008 WHO classification criteria.[3] The clinical history of each patient was reviewed as follows: Age at diagnosis, gender, location of the tumor, staging, and treatment regimens. The staging was decided according to the system proposed by Murphy and Hustu and modified by Magrath.[3] None of the patients had primary or acquired immunodeficiencies.

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Construction of tissue microarray (TMA) was conducted as previously described. Briefly, all specimens were sectioned for H and E staining and assessed for morphology. The representative regions of tumor were marked on the H and E stained slides and identified on the corresponding tissue blocks. Tissue cylinders of 2 mm diameter were punched from the marked regions of each block and incorporated into a recipient paraffin block. The recipient paraffin blocks (TMA) were then baked at 56°C for 10 min before sectioning.

Immunohistochemistry
Immunohistochemical studies were performed on 4 μm thick paraffin sections cut from TMA blocks using the streptavidin-biotin peroxidase complex method, and when the staining result was not satisfactory, whole sections from the original tumor block were stained. The antibodies used were those raised against the following antigens: CD3, CD10, CD20, CD79a, TdT, BCL6, BCL2, MUM1, and Ki-67. Details of the antibodies including sources, dilutions, and antigen retrieval conditions are listed as previously reported. According to the expression of MUM1 and BCL-6, BL were subclassified into four groups: Germinal center (GC) (MUM1+/BCL-6+), late-GC (MUM1+/BCL-6−), post-GC (MUM1+/BCL-6+) and mantle cell-like (MUM1−/BCL-6−). Interphase FISH was performed on paraffin sections using an EBV-encoded RNA (EBER) peptide nucleic acid detection kit (Tape Bio-Technology Co. Ltd., Fujian, China). Cases were interpreted as EBV positive if more than 10% of the tumor cells showed nuclear staining with yellow-brown granules. Tissue specimens from patients with EBV positive Hodgkin lymphoma were used as positive controls, and negative controls were stained with phosphate buffered saline instead of the probe. Fluorescence in situ hybridization for c-myc gene rearrangement
Fluorescence in situ hybridization (FISH) assays were performed on 4 μm thick unstained, probes used in this study was purchased from Vysis (Vysis, Gene Ltd., China). Interphase FISH was performed on paraffin sections as previously described. In each case, around 100–200 tumor cell nuclei were examined. Cut-off values were calculated as reported. Raji cell line was used as a positive control.

Follow-up and statistical analysis
Clinical follow-up data were available for 54 of the 92 patients. SPSS version 17.0 software (SPSS Inc., USA) was used to analyze, lymphoma-specific survival time was calculated by determining the time from the date of diagnosis to the date of death or last follow-up, and was analyzed using the Kaplan–Meier method. The χ²-test or two-tailed Fisher exact test was applied. A P < 0.05 was considered as statistically significant.

RESULTS

Clinical features
From 1984 to 2013, BL accounts for 38.98% of all lymphomas of children. Among the 92 cases, there was a male predominance in sex distribution (M:F = 3.38:1). The mean and median age at diagnosis was 4.97 and 5.50 years, respectively (ranged from 3 months to 14 years). Abdomen was the most common site (88 cases, 95.7%), followed by superficial lymph node (3 cases) and testis (1 case). In situ hybridization for Epstein-Barr virus
In situ hybridization for EBV was performed on the available paraffin sections using an EBV-encoded RNA (EBER) peptide nucleic acid detection kit (Tape Bio-Technology Co. Ltd., Fujian, China). Cases were interpreted as EBV positive if more than 10% of the tumor cells showed nuclear staining with yellow-brown granules. Tissue specimens from patients with EBV positive Hodgkin lymphoma were used as positive controls, and negative controls were stained with phosphate buffered saline instead of the probe.

Morphological features
Burkitt lymphomas showed a diffuse lymphoid infiltrate and exhibited a starry-sky pattern. The tumor cells were uniformly medium in size, with round nuclei and a few nucleoli in most cases. However, a mild degree of irregular nuclear contours was noted in some tumors.
Immunophenotype and Epstein-Barr virus status

All of the cases were positive for CD20 [Figure 3a] and CD79a and negative for BCL-2, CD3, and TdT. The positive expression rate of CD10, BCL-6 [Figure 3b], MUM1 and EBER [Figure 3c] were 95.7% (88 cases), 92.4% (85 cases), 22.8% (21 cases), 41.3% (38 cases), respectively, Ki-67 + ≥90% [Figure 3d]. According to the expression of MUM1 and BCL-6, BL were divided into GC group 65 cases, late-GC group 20 cases, post-GC Group 1 case and mantle cell-like group 6 cases. The expression of MUM1 was not associated with EBV infection status (P = 1.000).

Fluorescence in situ hybridization

C-myc gene rearrangement was detected in 94.6% (87/92) [Figure 4] by FISH. Five cases without c-myc gene rearrangement, according to morphological features and immunophenotype and without BCL-2 and BCL-6 gene abnormality diagnosed BL (date not shown).[4,7]

Treatment, follow-up and survival analysis

Clinical treatment information was assessment for 54 of 92 cases, the follow-up time ranging from 2 days to 120 months. Among the 54 cases, 21 patients received surgery alone, all patients died of tumor (the average survival time was 5.2 months, ranging from 2 days to 20 months). Totally, 33 patients received surgery and chemotherapy ([R]CHOP-like regimen and NHL-[BFM]-90 regimen), six patients died of tumor and associated disease (including four bone marrow relapse cases, one respiratory failure case and one acute tumor lysis syndrome case) with the average survival time 9.7 months (ranging from 4 to 18 months), 27 was still alive with the average survival time 63.4 months (ranging from 8 to 120 months), including nine polypoid cases and one Stage II case without timely treatment. For the 33 patients received surgery and chemotherapy, advanced clinical stages were correlated to the poorer survival of BL (P = 0.024). MUM1 positive expression in 3 of 6 died cases [Figure 5] (P = 0.076).

DISCUSSION

Burkitt lymphoma is one of the most common pathologic
types of malignant tumor in children and young adults.\[2,5\]

There was a male predominance in sex distribution, especially in children.\[8-10\] Most cases of sporadic BL presented with extranodal lesions such as abdominal masses,\[12,11,12\] which is usually at early stage of diagnosis and without more organ involvement because of intussusception, especially in polypoid patients in our study. The St. Jude staging system, which was intended for all histological subtypes and also included Stages I–IV (Stages I and II named limited stage, Stages III and IV named advanced stage), clinical staging varied according to the anatomic sites of involvement, clinical presentation and diagnosis level, most literature reported more than half BL patients presented at advanced stage.\[8,9\] Our finding is accordant with previously reported. Serum albumin level is an index of body nutrition, low serum albumin level may increase the risk of infection and decrease the tolerance of chemotherapy drugs, thus, serum albumin level was a prognostic marker of tumor.

Burkitt lymphoma is a highly aggressive NHL originated from the GC or post-GC B-cells.\[2]\ Most cases show positive expression of GC associated antigen CD10 and BCL-6.\[10,13\] MUM1 is a lymphocyte-specific member of the interferon regulatory factor family of transcription factor expressed in post-GC B-cells and plasma cells. The expression of MUM1 was controversial in BL, Tumwine et al.\[13\] reported 95 BLs from Uganda was negative expression, the next year, Gualco et al.\[10\] reviewed 222 cases and found MUM1 was positive in 43% of children BLs and 38% of adult cases. These results indicate that the expression of MUM1 is not an exclusionary marker for diagnosis of BL. The origin of tumor cell is associated with prognosis, MUM1 is a marker of late-GC and post-GC B-cells, it is a prognostic factor in many lymphomas, including follicular lymphoma and classical Hodgkin’s lymphoma.\[14,15\] survival curves showed nearly statistical significance between MUM1 expression and prognosis in our study. The significance and mechanism of MUM1 expression will be studied in the future.

Epstein-Barr virus is associated with many lymphomas, including BL and extranodal NK/T-cell lymphoma, nasal-type and so on.\[2,16\] EBV was detected in 41.3% of the BLs in our study, which is higher than Bi et al.\[10\] reported, lower than from South-eastern Brazil reported.\[17\] the frequency of EBV infection in sporadic BL varied according to geographical distributions.\[10,18\] and the relationship between EBV and sporadic BL is still not very clear.\[19\] According to immunoglobulin gene analysis, EBV-positive and EBV-negative BL revealed two distinct cells of origin, EBV-negative BL has a lower average mutation frequency than EBV-positive BL, indicating an origin in the early centroblast for sporadic EBV-negative BL, and EBV-positive BL corresponding to the late-GC B-cells that have begun the differentiation process into memory B-cells.\[19\] One may hypothesize that the higher expression of MUM1 may be related with high frequency of EBV infection. Unfortunately, EBV infection seems not to be the only factor explaining this higher MUM1 expression,\[5\] our finding supported this viewpoint.

More than 90% cases of BL showed a detectable c-myc gene rearrangement by FISH, which is a characteristic but not specific feature of BL.\[21\] In our findings, 94.6% patients showed c-myc gene rearrangement. In 5 cases of without c-myc gene rearrangement, according to morphological features and immunophenotype and without BCL-2 and BCL-6 gene abnormality diagnosed BL.\[19\] Because of the limit of laboratory conditions, the gene detection has not been carried out in many hospitals. Gonin et al.\[22\] found EBV-induced gene 3 (EBI3) was a practical diagnosis marker in BL, showed that EBI3 was not expressed in all BL cases, whereas it was expressed by over 30% of tumoral cells in nearly 80% of diffuse large BCL (DLBCL) cases, and DLBCL or BCL, unclassifiable, with features intermediate between DLBCL and BL (DLBCL/BL) cases with c-myc translocations have lower expression of EBI3. The results indicated EBI3 immunohistochemistry could be useful to discriminate BL from DLBCL, and to identify cases of DLBCL/BL or DLBCL with potential c-myc translocations. Besides, CSE1L and inhibitor of DNA binding-3 (ID3) overexpression was associated with the diagnosis of BL and signal transduction and transcription-3 with DLBCL, and enabled the identification of patients with DLBCL/BL who benefited from intensive chemotherapy regimen.\[23\]

The refinements in both the staging and response evaluation of children with BL have contributed to the improvements in treatment outcome observed in the past decades. Although NHL-BFM-90 chemotherapy regimens improve cure rates of up to 95% in limited stage and 80% in advanced stage,\[24\] advanced stage and tumor lysis syndrome is a mainly cause of death of the treating patients.\[25\] In addition to clinical parameter, molecular genetic abnormality was a key factor for prognosis of BL, such as 13q14.3 deletion was a poorer prognosis marker of BL.\[26\] In our study, 6 of 33 chemotherapy patients died of tumor and associated disease, 21 patients received surgical resection alone, all patients died of the tumor with the average survival of 5.2 months. Notably, many low stage cases that giving up treatment after surgery, the possible reasons may include, but not limited to: (1) Worried about poverty because of this disease; (2) Parents were lacking of associated medical knowledge. Hence, health education is essential for improving life quality and overall survival rates of patients with BL.

In conclusion, our study showed that the location and growth pattern and serum albumin level of BL were associated with biological behavior. Because of the limited cases, the significance and mechanism of MUM1 expression will be studied in the future. It is important for us to improve the diagnostic accuracy of BL, and to strengthen the management of the tumor to improve the prognosis in the future.

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