ORIGINAL ARTICLE

Morphological Study Remains the Mainstay in the Diagnosis of Small Round Blue Cell Tumours in Childhood

MUNEEZA KHALID, A.H NAGI, NAILA ATIF, *NASIR U DIN, AFZAL SHEIKH*

ABSRACT

Aim: To study the importance of morphology in face of attractive new technology.
Materials and methods: All tissue samples were fixed in formalin, embedded in paraffin and routinely stained with H and E, PAS for glycogen, silver for nerve fibers, PTAH for strap cells, CD 3, CD 20, and LCA for lymphoma. Reactions were preformed with appropriate positive and negative controls.
Results: A total of 100 cases were included ,among these 29 were diagnosed as neuroblastoma,23 were Wilm's tumour, 14 were Non Hodgkin's lymphoma,9 were rhabdomyosarcoma, 8 were Ewing's sarcoma, 6 were medulloblastoma,5 were lymphoblastic lymphoma, 4 were retinoblastoma and 2 were Burkitt's lymphoma.
Conclusion: Round blue cell tumours were more common in males in the first two decades of life. There is a significant association between presenting complaints and histological type of tumours. An association was also observed between histological type and morphological features. LCA CD 20 and CD 3s were helpful in confirming the diagnosis of lymphoma. In addition special stains such as PAS, silver and PTAH were very useful in differentiating small round blue cell tumours.
Keywords: Small round blue cell tumours, Wilm's tumour, rhabdomyosarcoma

INTRODUCTION

The predominant solid tumours of childhood are the small round blue cell tumours (SRBCT) which comprise 30% of childhood malignancies Cohn et all (1991)[1]. These include neuroblastoma, retinoblastoma, Wilm's tumour, Ewing's family of tumours, rhabdomyosarcoma, lymphoma, medulloblastoma and desmoplastic round blue cell tumours. They are so named because they are similar in appearance on routine morphology. Typically on histopathological examination they are undifferentiated, uniform, small round cells with hyperchromatic densly blue nuclei because of high amounts of DNA, hence the name "Round blue cell tumors" Pizzo et all(1997)[2]. The current approach to the diagnosis and classification of these tumours is to employ a multimodality approach. This includes examination by light microscopy, immunphenotyping, by immunhistochemistry methods, cytogenetics and molecular genetics. With progressive use of immunohistochemistry knowledge of crossreactivity, technical problem cytogenetics and electron microscopy not available at every institution .However, histologically, these tumours show a significant variation in terms of biological patterns, behavior, prognosis and treatment strategies. Currently there exists no single biological or chemical test that can precisely distinguish SRBCT of childhood. It therefore seems worthwhile to do detailed morphological study to assess the added value of morphology to determine their subclasses Granja et al (2005)[3]. In the present study we have examined 100 cases of round blue cell tumours collected from Children Hospital and Mayo Hospital between 2007-2009. We have attempted to expand the identification of round blue cell tumours into subclasses.

Departments of Pathology, University of Health Sciences and Pediatric Surgery, Institute of Child Health, Lahore

Correspondence to Dr. Muneeza Khalid, Assistant Professor (Histopathology), Central Park Medical College, Lahore E-mail: muneezanajeeb@hotmailcom

MATERIALS & METHODS

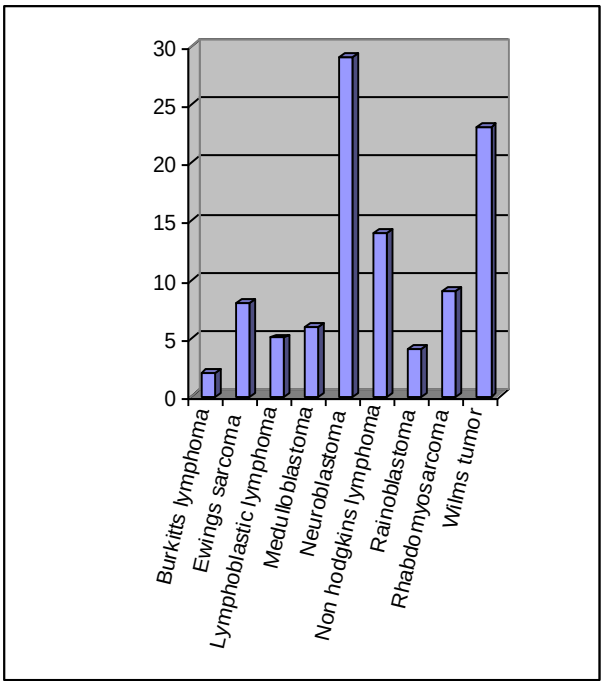
A descriptive study was preformed on 100 cases diagnosed as round blue cell tumours between 2007-2009 at Children Hospital 85 cases, Mayo Hospital 15 cases. Histopathological diagnosis were performed keeping in view the clinical features and the morphology. Patients with other co morbid cancers were excluded. In every case, paraffin-embedded tissue blocks for histological evaluation, special stains and Immunohistochemistry were used. The present study was approved by the ethical committee of the Children Hospital, Mayo Hospital and University of Health Sciences. All tissue samples were fixed in formalin, processed for wax embedding and stained using H+E routine stain followed by if required PAS for glycogens, silver for nerve fibers, PTAH for strap cells.

Histological section (5-8m thick) was obtained from the paraffin embedded tissue specimens they were deparaffinised in xylene and rehydrated. The cases of lymphoma were reacted with mouse monoclonal antibody provided by novocastra in liquid using an, avidin-biotin-peroxidase complex. Reactions were performed with appropriate positive and negative controls.

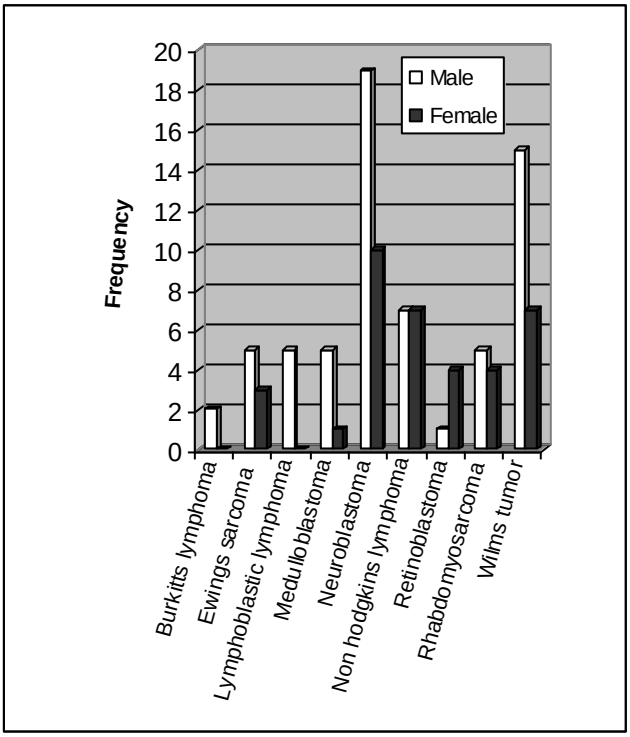
The data was entered and analysed using SPSS 17.0, mean S.D was given for quantitative variables. Frequencies percentage and graphs are given for qualitative variables. Pearson chi square and fisher exact test were applied to observe association between qualitative variables.

RESULTS

Among the 100 cases ,29 were found to be neuroblastoma,23 were Wilm's tumour, 14 were Non Hodgkin's lymphoma,9 were rhabdomyosarcoma, 8 were Ewing's sarcoma ,6 were medulloblastoma,5 of lymphoblastic lymphoma as shown in the flow chart i.e figure 1.A detailed morphological examination was carried in all patients with special emphasis on the histochsemistry and IHC stains.



In Burkitts lymphoma group, both patients were males in Ewing's sarcoma group, 5(62.5%) were males and 3(37.5%) were female making a M:F ratio of 1.6:1. Among the lymphoblastic lymphoma group all 5 cases were males (100%) making a M:F ratio of 1:0. In the medulloblastoma group 5 were male (83.3%) and 1(16.7%) was female. In neuroblastoma 19(65.5%) were males and 10(35.5%) were female making an M:F ratio1.9:1. In retinoblastoma groupi.e 4 case, one(25%) was male and 3(75%) were female making a male to female ratio of 1:3.Among the Rhabdomyosarcoma 5(55.5%)were males and 4(54.6%) were females making a M:F ratio 1.2:1 .In Wilm's tumour 15(65.2%) were males and 8(34.7%) were females with a male to female ratio of 1.8:1 whereas in Non Hodgkin's lymphoma 7 cases(50%) were males and7(50%)were females making an M:F ratio 1:1.



DISCUSSION

The diagnosis of round cell tumours based on histopathology alone is often challenging, because an accurate diagnosis is important in determining prognosis and treatment. Tumour diagnosis has undergone a change over the past two decades .It includes immunophenotyping and genetic analysis .In Pakistan, where histopathology is still evolving as a science and most centers lack immunohistochemistry and genetic facilities, it is essential that more attention paid to detailed morphological examination and special histochemical stains. In the present study 100 cases of RBCT were studied. Neuroblastoma constituted 29% of all the malignant tumours and there is slight sex predominance in male to female of 1.2:1 as seen in study report by Riley et al (2004). In the present study the M:F with the median age at diagnosis was 4.34. Most patients present with may

be accompanied by constitutional symptoms of fever, weight loss and malaise. Among 29 children, 24(82.2%) came with abdominal mass 13(44.8%) presented with abdominal distention 13(44.8%) had fever and 7(24.1%) showed purpura. On Ultrasonography typical findings are a large irregularly shaped, hetrogenous mass in the adrenal region that had areas of necrosis, haemorrhage and calcification. Aetiology is unknown but research has focused on maternal and paternal exposures prior to conception and during gestation. Kerr et al (2000)[6,5]. On morphological examination we observed that 24(82.8%) cases were small cell size and 5(17.2%) were medium cell size with 22(75.9%) having scant cytoplasm and 6(20.7%) showed moderate cytoplasm. The nuclei were round in 14(48.8%) cases and oval in 15(51.7%). Kusher et al (2004). In his study observed that diagnosis of neuroblastoma is the characteristic histological finding- that is neural and ganglion features. Similar to the results observed by Wei et al (1990) reported that 7 out of 12 had Homer Wright rosettes. All the 29 cases in our series had Homer Wright rosettes hence we think that acidophilic fibrillary intercytoplasmic background is most useful diagnostic feature.

Wilm's tumour is renal tumour forms 23% of all the malignant tumours of childhood. Twenty three cases of Wilm's tumour were studied with special reference to their morphological variations. Male/female ratio was 1.8:1; the mean age being in years. There was a male preponderance in the study, an observation identical to that of Sharma et al (1995) though some other coworkers have reported more or less an equal involvement of both sexes. Among the 23 cases diagnosed as Wilm's tumour 20(87.8%) presented with abdominal mass and 13 (56.6%) with abdominal distention. Among the 23 children, 14(60.9%) complained of haematuria. Morphological examination showed that 20(87%) cases had small cell size and 3(13%) had medium cell size with 17(73%) having scanty cytoplasm and 6(26.0%) having moderate cytoplasm. The nuclei were round in 14(60.9%) cases and oval in 9(39.1%). The classic triphasic pattern is present in the tumour when it contains blastemal, stromal, and epithelial components however, the cell types occur in varying proportions with some consisting of only biphasic or monophasic patterns. Our study showed that all the 23 contain blastemal component with 19(82.6%) having mesenchymal component and 12(52.2%) showing tubular component. Morphological study revealed triphasic pattern in 52.2%cases, biphasic (epithelial + stromal) in 82.6% and monophasic in none.

Embryonal rhabdomyosarcoma is the most common soft tissue sarcoma in children less than 15 years of age. It constituted 9% of all the malignant tumours of childhood. The embryonal subtype accounts for 60% of childhood rhabdomyosarcomas and is most frequently seen in the soft tissue of head and neck, with less common presentations in the genitourinary tract. In the present study 9 children were diagnosed as rhabdomyosarcoma. This study showed a male preponderance (M:F 1.2:1) similar to that reported in other studies (Ranjiv Sivanandan, Christina S. Kong, Michael J. Kaplan 2004). Among the 9 cases presented with nasal mass, 2(22.2%) showed aural mass and 2(22.2%) children had abdominal distention. In the present study all the cases were of embroynal type that is similar to another study (Mondal et al 2010) who reported that80% cases belonged to embryonal type. Differentiated rhabdomyoblasts have been described as strap cells, zigzag cells, and broken straws. Among the 9 cases, 3(33.3%) showed small cells, 6(66.7%) consisted of medium cells, 3(33.3%) showed scanty cytoplasm and 4(44.4%) contained moderate amount of cytoplasm. The nuclei were round in 14(60.9%) cases and oval in 9(39.1%). The strap cells and perivascular pseudorossettes were present in 7(77.8%) cases. PTAH used in our study highlighted cross-striations and confirmed our diagnosis

The most common ocular cancer in children is retinoblastoma that usually occurs before 5 years of age, constituting 4% of all the malignant tumours of childhood. Male/female ratio was 3:1; consanguinity was 8.97% and positive family history was present in 25%. In the present study all the four children presented with visual complaints, 3(75%) showed proptosis and 3(75%) some with ptosis. It is thought that retinoblastoma may have a hereditary pattern, though it frequently occurs without prior family history. Only one of the four cases included in our study had positive family history. Undifferentiated elements appear as collections of small, round cells with hyperchromatic nuclei; the differentiated cells include Flexner-Wintersteiner rosettes and fluerettes from photoreceptor differentiation. The morphology of 3(75%) of our cases showed small sized cells surrounded by scanty cytoplasm and 1(25%) had medium sized cells having moderate amount of cytoplasm. The nuclei were round in 3(75%) and oval in 1(25%) case. **Ewing's sarcomas** are found in children aged 10 to 20 years. It is slightly more common in boys (55:45 male: female ratio). The most common age at diagnosis is the second decade of life, although 20%–30% of cases are diagnosed in the first decade however in the present study M:F ratio was 1.6:1 and mean age was 7.75years Verrill et al (1997) showed that the 37 male and 22 female patients had a median age of 24 years.

Similarly Venkitaraman et al (2007) studied 19 patients, 12 males 7 females with median age 21 years. Lower limb was the most common primary tumour. Clinical presentation is usually dominated by local bone pain and a mass. In the present study 8 among 100 cases were diagnosed as Ewing's sarcoma. 4(50%) presented with bone mass, 3 (37.5%) had fever, 1(12.5%) child complained of headache, 1(12.5%) had pelvic mass and another 1(12.5%) had abdominal distention. Jambhka et al. (2006) in their study reported that 19(59.38%) of 32 cases presented with primary bone tumours; 12 of the 19 involved the lower extremities and 7 upper extremities. Soft tissue tumours (extra osseous) were seen in 13(40.62%) of 32 cases[7,8]. The morphological examination 6(75%) showed that the cells were small in 2(25%) they were medium where 6(75%) had scant cytoplasm and 2(25%) showed moderate cytoplasm. The cells have scanty, faintly eosinophilic to amphophilic cytoplasm, indistinct cytoplasmic borders, and round nuclei with evenly distributed, finely granular chromatin and inconspicuous nucleoli. The nuclei were round in 5(62.5%) cases and oval in 3(37.5%) cases. A useful technique to confirm the presence of Ewing's sarcoma is a strongly positive PAS stain that is sensitive to diastase treatment. All the 8 biopsies were PAS positive for cytoplasmic granules confirm the diagnosis of Ewing's sarcoma. These findings are in accordance with those reported by Telles et al (1978).

Medulloblastoma is the most common malignant brain tumour of childhood accounting for 40% of all posterior fossa tumours. In our study among the 100 children, 6 were diagnosed as having medulloblastoma. The M:F ratio was 5:1 and mean age of 6.25 similar the to study reported by Park et al (2007). Among our cases, 5(83.3%) presented with headache and increased head circumference. Three (50) had disturbed gait and 2(33.3%) complained of parasthesias. Medulloblastoma on the basis of morphology is divided into two types i.e., classic and desmoplastic. Among 87 classical and 43 desmoplastic variants of MB were studied by Praminik et al (2003) with respect to clinical and histological characteristics.[82] In the present study 4 were classical and 2 were of desmoplastic type. Morphological 5(83.3%) of the 6 cases showed small sized cells and nuclei having scanty cytoplasm and 1(16.7%) was medium sized with moderate amount of cytoplasm. The nuclei were round in 4(66.7%) biopsies and oval in 2(33.3%).

The third rather common childhood cancer is **lymphoma**; it accounts for 10% of all childhood cancers. In the present study among 100 cases, 14 were diagnosed as Non Hodgkins lymphoma (NHL), 5 as lymphoblastic lymphoma and 2 as Burkitt's lymphoma. Although NHL increase has been observed for both genders in lymphoid cancers it is more frequent in males than females. In the present study, however M:F ratio was 1:1 in non Hodgkins lymphoma. All cases of lymphoblastic lymphoma and Burkitt's were males, out of the 14 children diagnosed as NHL 7(50%) had lymphadenopathy, 4(28.6%) had fever and 6(42.9%) presented with abdominal mass. In a clinicomorphological study on 34 consecutive cases of Lymphoblastic Lymphoma (LBL) in children showed, that the highest prevalence age group was 5-10 years, with a male: female ratio of 2.7:1. On histological examination, architecture was effaced in 85.3% cases. The neoplastic cells were medium sized with round to oval nuclei showing fine, delicate chromatin. The cytoplasm was scanty and weakly basophilic. Among the 5 cases of lymphoblastic lymphoma 4(80%) presented with lymphadenopathy and 4(80%) with abdominal mass whereas fever and abdominal distention were presented in 3(60%) of the 5 cases. As regards morphological features 8(57.1%) cases had small cells and 6(42.9%) had medium cells with 9 having scanty cytoplasm and 5(35.7%) with moderate amount of cytoplasm. The nuclei was round in 10(71.4%) case and oval in 4(28.6%) among the five cases of lymphoblastc lymphoma all had small nuclei with 3(60%) having scanty cytoplasm and 2(40%) showed moderate amount of cytoplasm. Blood vessel permeation was observed in 2(40%) whereas 1(20%) case showed starry sky pattern. The 2 children who were diagnosed as Burkitt's lymphoma both had lympadenopathy and abdominal mass with 1(50%) had fever. Blood vessel permeation was seen in both and starry sky pattern was present in one. Immunostaining was carried out on 25 cases with suspicion of lymphoma and 21 were positive for lymphoma. All 25 cases were LCA positive 19 showed membrane staining with CD 20 and 2 showed positive membrane staining with CD 3.

In conclusion we feel that SRBCTs are diagnosable by optical microscopy except for NHL a help from lymphoma markers is required. In addition some histochemical stains are also helpful.

Acknowledgments: We acknowledge our gratitude to Children Hospital and Mayo Hospital Lahore for fully supporting this research activity.

REFERENCES

1. Cohn SL. Diagnosis and Classification of Small Round Cell Tumours of Childhood. AJP 1999; 155:11-5
2. Pizzo, P.A. Principles and practice of Paediatric Oncology. (Lipponcott-Raven, Philadelphia 1997)
3. Granja NM, Begnami MD, Bortolan J, Filho AL, Schmitt FC. Desmoplastic small round cell tumours: Cytological and immunocytochemical features. Cytojournal. 2005 Mar; 18 2(1):6.

4. Marinsek PZ. Difficulties in diagnosing small cell tumours of childhood from fine needle aspiration cytology samples. Cytopath 2008; 19;67-79
5. Richard D. Riley, David Heney, David R. Jones, Alex J. Sutton, Paul C. Lambert, Keith R. Abrams, Bridget Young, Alan J. Wailoo and Susan A. Burchill. A Systematic Review of Molecular and Biological Tumor Markers in Neuroblastoma; Clinical Cancer Research, January 2004 10; 4
6. Kerr MA, Nasca PC, Mundt KA, Michalek AM, Baptiste MS, Mahoney MC. Parental occupational exposures and risk of neuroblastoma: a case-control study (United States). Cancer Causes Control. 2000 Aug; 11(7):635-43.
7. Kushner BH, Gilbert F, Helson L. Familial neuroblastoma. Case reports, literature review, and etiologic considerations. Cancer. 1986 May 1; 57(9):1887-93.
8. Sharma S, Nath P, Srivastava AN, Singh KM.Wilm's tumour:a clinicopathologic study with special reference to its morphologic variants.Indian J Pathol Microbiol.1995 Jan; 38(2):55-62.
9. Sivanandan R, MD, FRCS; Christina S. Kong, MD; Michael J. Kaplan, MD; Willard E. Fee, Jr, MD; Quyhn Thu-Le, MD; Don R. Goffinet, MD. Laryngeal Embryonal Rhabdomyosarcoma; Arch Otolaryngol Head Neck Surg. 2004; 130:1217-1222
10. Mondal PK, Pal I, Misra S, Biswas S, Bera SP. Rhabdomyosarcoma of nose, nasopharynx and paranasal sinuses. Indian Journal of Otolaryngology and Head & Neck Surgery 2009; 61(4): 317-9.
11. Verrill MW, Judson IR, Harmer CL, Fisher C, Thomas M, Wiltshaw E. Ewing's sarcoma and primitive neuroectodermal tumor in adults: J Clin Oncol. 1997; 15:2611–2621
12. Venkitaraman R, George MK, Ramanan SG, Sagar TG. A single institution experience of combined modality management of extra skeletal Ewing's sarcoma. World J Surg Oncol. 2007 Jan 11; 5:3.
13. Jambhekar, Nirmala A, Bagwan, Izhar N, Ghule, Prachi, Shet, Tanuja M, et al. Comparative Analysis of Routine Histology, Immunohistochemistry, Reverse Transcriptase Polymerase Chain Reaction, and Fluorescence In Situ Hybridization in Diagnosis of Ewing Family of Tumors: Arch Pathol Lab Med. 2006; 131:1813-1818
14. Telles NC, Rabson AS, Pomeroy TC. Ewing's sarcoma: an autopsy study. Cancer 1978; 41:2321-2329.
15. Park HJ, Nam BH, Lim HS, Shin HY, Hah JO, Kang HJ, et al. Outcome of Multicenter Study for Korean Children with Medulloblastoma. Clin Pediatr Hematol-Oncol. 2007 Oct; 14(2):167-75
16. Pramanik P, Sharma MC, Mukhopadhyay P, Singh VP, Sarkar C. A comparative study of classical vs. desmoplastic medulloblastomas. Neurol India 2003; 51:27-34.
17. Tahir M, Ali L. Lymphoblastic Lymphoma in Children, J Rawal Med Coll Dec 2000; 4(1-2):27-9.