Article published online: 2021-07-19

ORIGINAL ARTICLE

Morphometric evaluation and clinical correlations in pediatric malignant small round cell tumors

Cherry Bansal,
Archana Gupta[1],
Archana Kumar[2],
Anand Srivastava

Department of Pathology, Era's Medical College and Hospital, Departments of [1]Pathology and [2]Pediatrics, King George Medical University, Lucknow, Uttar Pradesh, India

Address for correspondence:
Dr. Cherry Bansal,
Associate Professor,
Department of Pathology, Era's Medical College and Hospital,
Hardoi Road, Lucknow - 226 003,
Uttar Pradesh, India.
E-mail: drcherrybansal@gmail.com

ABSTRACT

Aims: Nuclear size increases in malignant tumors and reflects DNA content, ploidy and proliferation index. Present study investigated if the nuclear morphometry could differentiate histomorphologically similar paediatric malignant small round cell tumors on hematoxylin and eosin stained sections for diagnostics in a resource poor setting. **Settings and Design:** All the consecutive malignant pediatric tumors received in Pathology Department from other faculties of King George's Medical University and also those referred directly to Pathology Department from other hospitals of city/other cities during 3 years period were recorded. **Materials and Methods:** Morphometric analysis was done in 22 confirmed (by higher ancillary techniques) but histomorphologically difficult to differentiate round cell tumors. All sections were analyzed by cell images from six different areas, using Leica Q win 500 images software. **Results:** Nuclear measurements were obtained for retinoblastoma (RB) (nine cases), neuroblastoma (five cases), Wilms tumor (WT) (three cases), rhabdomyosarcoma (three cases), malignant hemangiopericytoma (one case) and non-Hodgkin lymphoma (one case). Among the RBs, maximum mean nuclear area percent (24.93) was seen in a case with nerve involvement and metastasis, followed by cases with only nerve involvement (21.60) and smallest area (16.57) was in non-nerve involving, nonmetastatic cases. All five cases of neuroblastoma had almost similar mean nuclear area percent (18.05-18.29). WT case with metastasis had higher nuclear area (21.25) than nonmetastatic (19.47). Amongst all the tumors, minimum value (14.93) was seen in malignant hemangiopericytoma. **Conclusion:** Morphometric evaluation in paediatric malignant round cell tumors have generated useful data, and needs further multicentric confirmation for implementation.

Key words: *Histopathology, morphometry, pediatric, small round cell tumors*

INTRODUCTION

Small round cell tumors (SRCTs) are characterized both cytologically and histologically by predominantly small, round to oval and relatively undifferentiated cells. They constitute an overwhelming majority of childhood malignancies. This group of malignancies include retinoblastoma (RB), neuroblastoma, hepatoblastoma, nephroblastoma, rhabdomyosarcoma (RMS), small cell anaplastic carcinoma, Ewing sarcoma peripheral neuroectodermal tumor, desmoplastic SRCT and non-Hodgkin lymphoma (NHL). SRCTs often pose a diagnostic challenge because of the morphologic similarities, especially when they are poorly differentiated.[1-5] To cut short the differential diagnoses list, and to arrive at a definite diagnosis, diagnostic accuracy of SRCTs can be enhanced along with one or more of the ancillary techniques[6] such as special stains (cytochemistry), immunocytochemistry,[7,8] electron microscopy,[9] morphometry,[10] tissue culture, DNA ploidy, karyotype and molecular analysis.[11-13] In the present study, we have worked out the role of nuclear morphometry to differentiate histomorphologically similar paediatric malignant SRCTs in hematoxylin and eosin (H and E) stained sections for using in a poor resource country. Majority of histopathology laboratories of a developing country do not imply auxiliary techniques, so the present study investigated the feasibility of precise diagnosis based on histomorphology and morphometry.

Access this article online

Quick Response Code:

Website: www.ijmpo.org

DOI: 10.4103/0971-5851.144987

MATERIALS AND METHODS

All the consecutive malignant pediatric tumors received in Pathology Department from other faculties of King George's Medical University and also those referred

directly to Pathology Department from other hospitals of city/other cities during 3 years period were recorded. Out of a total of 83 cases, 22 cases were selected to perform morphometric analysis. These 22 cases of SRCT elude identification on light microscopy (H and E stained sections) alone. Histologically, tissue sections showed relatively undifferentiated cells which were predominantly small round to oval. These archived cases were already worked up cases with application of special stains (cytochemistry), immunohistochemistry and molecular techniques as per the individual case diagnostic requirement. Morphometric analysis was done on representative areas of H and E sections with Leica Q Win 500 image analysis software (Leica GMBH, West Germany). For a single case, images were captured from a minimum of six different areas. Nuclei were tagged blue and with Acquisition and Analysis software; parameters such as nuclear area, nuclear diameter, nuclear perimeter, area fraction, area fill count, percent area etc., were calculated. Final results were obtained from the mean of percent areas of different images for a particular case and results were compared and analysed.

RESULTS

Patient age varied from 6 months to 13 years with a mean age of 6 years. Male children (16) were more than female (6). Morphometric analysis was obtained for RB (nine cases), neuroblastoma (six cases), Wilms tumor (WT) (three cases), RMS (three cases), malignant hemangiopericytoma (one case) and NHL (one case). Nuclear area percent varied from 14.93 to 24.93 with a mean value of 18.91. Amongst the RBs, maximum mean nuclear area percent (24.93) was seen in a case with nerve involvement and metastasis, followed by cases with only nerve involvement (21.60) and smallest area (16.57) was in non-nerve involving, nonmetastatic case. Three cases of RB with optic nerve involvement (case number 5, 8, 9) showed significant increase in mean nuclear area percent. Neuroblastoma cases (5) had similar results for mean nuclear area ranging from 18.05 to 18.29. WT case with metastasis had maximum nuclear area (21.25) than nonmetastatic (19.47). Case number 16 was diagnosed as poorly differentiated WT with blastema predominance, but morphometry yielded results in almost similar range. Embryonal RMS nuclear area (19.29) was more than alveolar RMS (18.00). Amongst all the tumors, minimum value (14.93) was seen in malignant hemangiopericytoma. Only one case was of NHL and no definite conclusion could be withdrawn to compare it with. Individual case diagnosis with details of age, sex and morhometric analysis is summarised in Table 1.

Table 1: Results of morphometry in 22 cases of pediatric SRCTs

Case number	Age/sex	Final diagnosis	Mean nuclear area %
1	6 months/male	RB	16.44
2	1.5/male	RB	16.34
3	3/male	RB	16.90
4	2.5/female	RB	16.50
5	2.5/female	RB with optic nerve involvement	19.52
6	2.5/male	RB with optic nerve involvement and lymph node metastasis	24.93
7	3/male	RB	16.70
8	3/male	RB with optic nerve involvement	23.02
9	5/male	RB with optic nerve involvement	22.28
10	3/male	Neuroblastoma	18.05
11	3/male	Neuroblastoma	18.68
12	4/male	Neuroblastoma	18.60
13	5/male	Neuroblastoma	18.10
14	5/male	Neuroblastoma	18.29
15	2.5/male	Wilms tumor with ureter infiltration	19.73
16	5/male	Wilms tumor; poorly differentiated	19.21
17	8/male	Wilms tumor with lymph node metastasis	21.25
18	3/female	Embryonal rhabdomyosarcoma	19.19
19	4/female	Embryonal rhabdomyosarcoma	19.39
20	13/female	Alveolar rhabdomyosarcoma	18.00
21	11/male	NHL	20.03
22	13/female	Malignant hemangiopericytoma	14.93

SRCTs – Small round cell tumors; RB – Retinoblastoma; NHL – Non-Hodgkin lymphoma

DISCUSSION

Morphometry is a type of quantitative analysis[14-17] and have been applied for a number of varied sites like breast, gastrointestinal tract, acute leukemias etc.[18-20] Childhood malignancies are rare, but those that occur often have the appearance of primitive "small round blue cell tumors" and pose a diagnostic challenge to the pathologist. Accurate tissue diagnosis is imperative in the group of tumors lumped under the category of malignant SRCT, because the treatment and management of these tumors is different.[21]

In the present study, malignant SRCTs in pediatric population irrespective of the region involved were included. Male children outnumbered female (16:6)

and majority cases were of RB (40.9%) followed by neuroblastoma (22.7%). Morphometric analysis on SRCTs by Brahmi *et al*. included Ewing's sarcoma (32.7%) and neuroblastoma (18%).[10] Ravindra and Kini studied morphometry of SRCTs in the region of the kidney and found WT (58%) was in majority with neuroblastoma (27.7%) being the second most common.[22] Kazanowska *et al*. studied in particular childhood RMS with maximum cases of embryonal RMS (60.2%) and concluded nuclear morphometry is a useful tool in the assessment of children with RMS.[23]

The available literature reveals that most of the researchers who worked on SRCTs have applied morphometry on aspiration smears,[10,20] investigated a particular differential of SRCT[22-26] and have also included adult subjects in addition to pediatric subjects.[10,22,23] Studies considering SRCTs in only pediatric subjects and applying morphometry on histopathology sections are few.[18,19,24,25,27]

The present study had the highest maximum mean nuclear area percent in a case of RB with nerve involvement and metastasis (24.93) and cases with nerve involvement had higher values than non-nerve involving. Brahmi *et al*. concluded that RB group of tumors showed the highest mean nuclear area and RB could successfully be differentiated from all other Materials Research Centers and Teams (MRCTs) with the help of morphometry.[10] Intratumoral heterogeneity of MYCN in neuroblastomas is rare.[28] Though, from our data we found that mean nuclear area percent was higher in cases of nerve involvement and metastasis but taking into consideration the overall mean values of RB, no significant difference was found from other categories.

We observed embryonal RMS nuclear area (19.29) was more than alveolar RMS (18.00). Brahmi *et al*. concluded that RMS had the highest mean convex area and RMS could be differentiated from all other MRCTs with the help of morphometry.[10]

CONCLUSION

In the present study, morphometric evaluation of pediatric SRCTs show that within a tumor type, the mean nuclear area percentage increases with tumor aggressiveness (i.e., lymphnode metastasis or nerve invasion). However, the evaluation did not generate any specific mean nuclear area range which could classify and categorize the differentials of SRCTs. Morphometric analysis in pediatric malignant SRCTs needs further multicentric confirmation studies. These studies can throw light on the categorization and prognosis of tumors provided the evaluation is done at specialized centres where specific tumors of one type are encountered, treated and followed-up.

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How to cite this article: Bansal C, Gupta A, Kumar A, Srivastava A. Morphometric evaluation and clinical correlations in pediatric malignant small round cell tumors. Indian J Med Paediatr Oncol 2014;35:267-70.
Source of Support: Nil, **Conflict of Interest:** None declared.