Clinical Research

Wilms' tumor 1 protein expression in embryonal and alveolar rhabdomyosarcoma and its association with clinical prognostic factors: a cross-sectional study

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ABSTRACT

BACKGROUND Embryonal rhabdomyosarcoma (ERMS) and alveolar rhabdomyosarcoma (ARMS) are the two major histological types commonly found in the pediatric population, which have different morphology and genetic profile. Wilms' tumor 1 (WT1) is an antigen highly expressed in solid tumors, including rhabdomyosarcoma, and a potential immunotherapy target. Only a few studies have attempted to determine WT1 expression in rhabdomyosarcoma. This study was conducted to demonstrate WT1 expression in ERMS, ARMS and associate it with established prognostic factors.

METHODS A cross-sectional study was conducted at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta using archival data from January 2011 to December 2017. 30 from 102 ERMS cases and 16 from 28 ARMS cases were included in this study. Data of age, tumor size, and location were collected. All cases were stained by WT1 immunohistochemistry. The expression was assessed semiquantitatively using histoscore (H-score) formula. An independent *t*-test was used to compare WT1 expression between ERMS and ARMS. Correlation analysis was used to evaluate the relationship between WT1 expression and prognostic factors.

RESULTS All ERMS and ARMS cases expressed WT1 in diffuse, moderate to strong staining. ERMS show higher WT1 expression than ARMS (H-score 179.9 *versus* 149.5) (p = 0.014). Strong WT1 expression mostly found in patient age <20 years and non favourable location. Moderate WT1 expression mostly found in cases with tumor size >5 cm.

CONCLUSIONS WT1 expression was higher in ERMS cases than in ARMS cases, which the expressions were similar in different age, tumor size, and location groups.

KEYWORDS alveolar, embryonal, prognostic factor, rhabdomyosarcoma, Wilms tumor

Rhabdomyosarcoma (RMS) is a malignant mesenchymal tumor with skeletal muscle differentiation that occurs more often in children and adolescents.[1,2] The incidence of RMS has been reported to be approximately 5% among all malignant tumors in children and <1% among all malignant tumors in adults. RMS comprises 50% of soft tissue sarcoma in children and adolescents.[3] Embryonal RMS (ERMS) and alveolar RMS (ARMS) have been reported to be more common than spindle cell or sclerosing RMS and pleomorphic RMS.[2,4]

The ERMS has bimodal age distribution.[2,5] They are usually located within the head and neck, genitourinary, and other locations. Compared with ARMS, only a few cases of ERMS occur in the extremities. Moreover, there is a loss of heterozygosity on chromosome 11p15.5.[5,6]

Meanwhile, ARMS occurs in the older age group, more frequently in adolescents and young adults. It generally occurs in the extremities. ARMS has a more aggressive biological behavior, can metastasize and appears as a rapidly growing and expansile mass in the extremities. ARMS consists of two histological subtypes, classic and solid.[6,7]

Studies in RMS patients have been conducted to improve therapeutic success and reduce the side effects of targeted therapy and immunotherapy.[8–11] According to National Cancer Institute pilot project of translational research on cancer vaccines, Wilms' tumor 1 (WT1) antigen had the highest tumor antigenic potential.[10] The WT1 gene is located on chromosome 11p13 and encodes transcription factors involved in normal embryogenesis, development of the urogenital system, spleen, mesothelium, smooth muscle, and some part of the central nervous system.[12] WT1 plays a role as a tumor suppressor gene in Wilms' tumor and as an oncogene in colorectal carcinoma, breast carcinoma, and brain tumors.[13–17]

The expression of WT1 is positive in fetal muscles and RMS but negative in normal adult skeletal muscles. WT1 is believed to have an oncogenic role in RMS.[18–20] A study found a stronger intensity of WT1 expression in ARMS than in ERMS.[13] The expression of WT1 was found to be associated with some prognostic factors and correlated to the grade of RMS according to *Federation Nationale des Centres de Lutte Contre le Cancer* and American Joint Committee on Cancer (AJCC) in various sarcomas but not specific to ARMS and ERMS.[18] This study was aimed to compare the expression of WT1 in ERMS and ARMS and to associate the expression of WT1 with its clinical prognostic factors. It is expected that the results of this study could provide information about WT1 expression in ERMS and ARMS and to explore the possibility of applying immunotherapy to these sarcomas.

METHODS

This cross-sectional study was conducted at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, and Cipto Mangunkusumo Hospital Medical Records Unit from February to May 2018. It was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: 0293/UN2.F1/ETIK/2018). This study included all cases of ERMS and ARMS recorded from January 2011 to December 2017. The inclusion criteria were all ERMS and ARMS cases diagnosed by histopathology and immunohistochemistry (IHC) with the morphological codes M8900/3, M8920/3, and M8901/3 and located throughout the body. The exclusion criteria were cases with incomplete clinical data and unavailable or inadequate paraffin blocks for further examination. Slides and forms were collected from the archive of the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital. Clinical data, including age, gender, location, tumor size, tumor incision limit, and clinical staging, were collected from the medical records. Tumor location can be classified into favorable and unfavorable according to prognostic significance. Favorable location including non-parameningeal head and neck, orbital, and paratesticular. Unfavorable location including extremities, bladder, prostate, parameningeal head and neck, retroperitoneal, and trunk.[21]

IHC staining was performed by a standard procedure, which were after deparaffinization with xylol and then blocking endogenous peroxide using 3% hydrogen peroxide in methanol for 30 minutes. Pretreatment with antigen retrieval procedure

Table 1. Clinical characteristics of ERMS and ARMS cases

Variables	Histological subtype, n (%)		*p*
	ERMS (N = 30)	ARMS (N = 16)	
Gender			**0.038***
Male	11 (36.7)	11 (68.7)	
Female	19 (63.3)	5 (31.3)	
Age (years)			0.742†
<20	22 (73.3)	11 (68.7)	
≥20	8 (26.7)	5 (31.2)	
Tumor size (cm)			0.208†
≤5	11 (36.7)	3 (18.7)	
>5	19 (63.3)	13 (81.2)	
Sample obtained by			
Biopsy	9 (30.0)	2 (12.5)	
Resection	21 (70.0)	14 (87.5)	
Location			**0.038***
Favorable	19 (63.3)	5 (31.2)	
Unfavorable	11 (36.7)	11 (68.7)	

ERMS=embryonal rhabdomyosarcoma; ARMS=alveolar rhabdomyosarcoma
*Chi-square test; †Fisher's exact test

in the decloaking chamber was done using tris-ethylenediaminetetraacetic acid (EDTA) at pH 9.0 and 95°C for 10 minutes. Blocking was performed using superblocks for 30 minutes. This was followed by incubation for 1 hour with WT1 primary antibody to N-terminus, clone 6F-H2 (Dako®, ready to use). The subsequent steps included incubation with anti-polyvalent UltraTek and then with UltraTek HRP, each for 10 minutes, followed by incubation with diaminobenzidine tetrahydrochloride for 1 minute. The slide was then counterstained with Mayer's hematoxylin for 30 minutes until the color turned blue. The final steps were dehydration with gradual alcohol concentrations and then clearing with xylol. The positive control used in this study was nephroblastoma (Wilms tumor).

The staining results were evaluated by two authors in a blinded manner. The staining was assessed semi quantitatively considering the intensity and percentage of stained tumor cells using the ImageJ computer program. The staining intensity was graded as negative/0, weak/+1, moderate/+2, and strong/+3. The percentage of stained tumor cells was evaluated using 500 tumor cells. Positive staining was defined by brown color staining of the tumor cell cytoplasm. The histoscore (H-score) formula was used for calculations, with the scores ranging between 0 and 300. The H-score results were further classified into the following four groups: negative (0–20), weak (21–80), moderate (81–180), and strong (181–300), based on a study conducted by Kim et al.[18]

Data analysis was performed using the SPSS software, version 21 (IBM) and a *p*-value <0.05 was considered to be statistically significant. Clinicopathological data were represented in the form of a frequency table. WT1 expression in ARMS and ERMS was analyzed statistically using an unpaired *t*-test or its alternative, and the correlation between WT1 expression and its prognostic factors were analyzed using Spearman's correlation and Mann-Whitney.

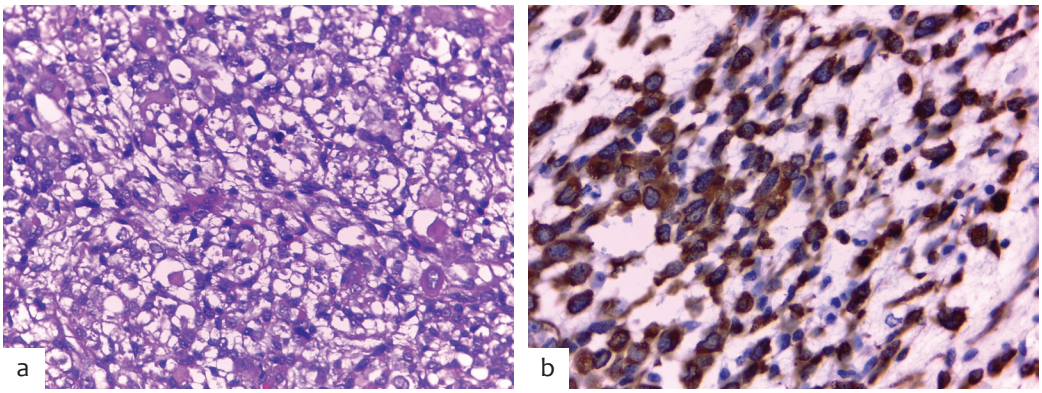

Figure 1. (a) Histopathology of embryonal rhabdomyosarcoma (ERMS), botryoid (H&E, original 400× magnification); (b) Immunostaining of Wilms' tumor 1 (WT1) in ERMS, botryoid, diffuse brown positive staining of tumor cells (WT1, original 400× magnification)

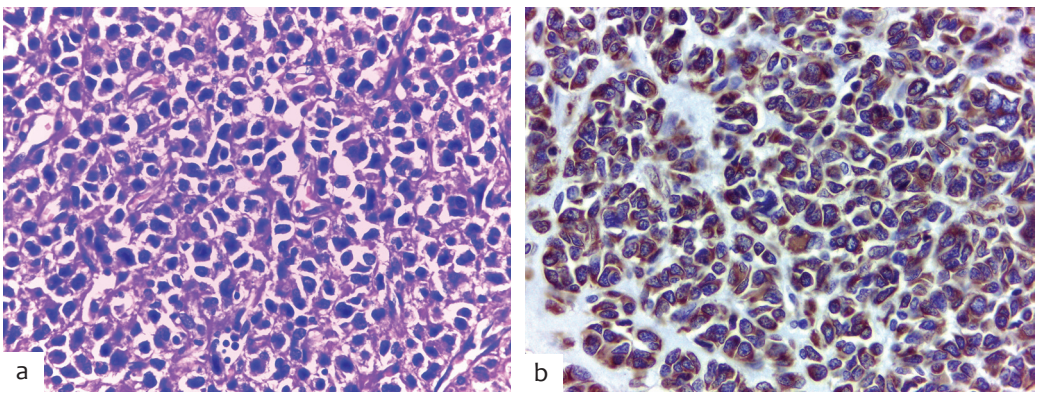

Figure 2. (a) Histopathology of alveolar rhabdomyosarcoma (ARMS), solid (H&E, original 400× magnification); (b) Immunostaining of Wilms' tumor 1 (WT1) in ARMS, solid. Diffuse brown positive staining in the cytoplasm of tumor cells (WT1, original 400× magnification)

Table 2. WT1 expression according to the histopathological subtype and distribution of WT1 H-score between ERMS and ARMS

	ERMS (N = 30)	ARMS (N = 16)	*p*
H-score, mean (SD)	179.9 (40.8)	149.5 (33.4)	0.014*
H-score category, n			
Negative (0–20)	0	0	
Weak (21–80)	0	0	
Moderate (81–180)	15	12	
Strong (181–300)	15	4	

WT1=Wilms' tumor 1; ERMS=embryonal rhabdomyosarcoma; ARMS=alveolar rhabdomyosarcomap; SD=standard deviation
*Unpaired independent *t*-test, $p = 0.014$; mean difference (95% confidence interval) = 30.4 (6.4–54.4)

RESULTS

A total of 130 RMS cases were recorded from January 2011 to December 2017. There were 102 ERMS and 28 ARMS cases. In ERMS group, 25 cases had inadequate/missing paraffin blocks for further IHC staining and 17 cases had incomplete clinical data. Hence, there were 60 ERMS cases which meet the inclusion criteria. Further, 30 ERMS cases were selected using random sampling technique. In ARMS group, 7 cases had inadequate paraffin blocks and 5 cases had incomplete clinical data. Hence, there were only 16 ARMS cases included in this study. The clinical characteristics of the ERMS and ARMS cases are listed in Table 1.

IHC staining on WT1 cytoplasm demonstrated diffuse positivity in both ERMS and ARMS cases. The staining was brown in appearance with varying intensities in the cytoplasm of tumor cells. There was no staining of tumor cell nuclei (Figure 1 and 2).

WT1 H-score was higher in ERMS than in ARMS ($p = 0.014$). WT1 expression according to the histopathological subtype is shown in Table 2. In the overall assessment of WT1 expression, all cases exhibited moderate positivity and strong positivity. There were no negative or weak positive cases. The distribution of moderate and strong positivity in ERMS and ARMS cases in each group of age, tumor size, and location are presented in Table 3.

Most patient age <20 years expressed strong staining of WT1. Patient age ≥20 years expressed moderate staining of WT1. Most tumor size ≤5 cm and >5 cm show moderate staining. Most tumor in favourable location express moderate staining and tumor in unfavourable location express strong staining. Strong WT1 expression mostly found in patient age <20 years and in non-favourable location. Most patient age ≥20 years, favourable location, tumor size >5 cm and ≤5 cm have moderate WT1 staining. The tumor size has median interquartile range (IQR) 8 (5.50) cm.

DISCUSSION

Studies about WT1 expression in malignancy, especially sarcoma, are still limited. This study showed WT1 was expressed in ERMS and ARMS cases. Studies conducted by Salvatorelli et al[14] and Magro et al[15] also demonstrated positive WT1 expression in ERMS and ARMS cases. Survival rate among RMS patient with WT1 expression was relatively lower than without WT1

Table 3. WT1 expression according to age, tumor size, and location

	ERMS, n (%) (N = 30)		ARMS, n (%) (N = 16)		*p**
	Moderate positivity (N = 15)	Strong positivity (N = 15)	Moderate positivity (N = 12)	Strong positivity (N = 4)	
Age (years)					0.301†
<20	8 (26.6)	14 (46.6)	8 (50.0)	3 (18.8)	
≥20	7 (23.3)	1 (3.3)	4 (25.0)	1 (6.2)	
Tumor size					0.303†
≤5 cm	5 (16.6)	4 (13.3)	3 18.8)	0 (0.0)	
>5 cm	10 (33.3)	11 (36.6)	9 (56.2)	4 (25.0)	
Location					0.084‡
Favorable	12 (40.0)	7 (23.3)	5 (31.2)	0 (0.0)	
Unfavorable	3 (10.0)	8 (26.6)	7 (43.8)	4 (25.0)	

WT1=Wilms' tumor 1; ERMS=embryonal rhabdomyosarcoma; ARMS=alveolar rhabdomyosarcoma
**p*-value for statistical analysis between WT1 expression with age, tumor size, and location, †Spearman's correlation test, ‡Mann–Whitney test

expression.[13] In other malignancies, such as leukemia and solid tumor, WT1 expression was also associated with worse prognosis. Moreover, WT1 vaccine has promising results in some clinical trials that showed good therapeutic response in children with leukemia and solid tumor.[9–11]

This present study also showed degree of WT1 expression was higher in ERMS than ARMS. Sotobori et al[20] reported different result that WT1 expression was significantly higher in ARMS than in ERMS, and it was associated with a worse prognosis. The higher WT1 expression in ERMS may be caused by some of paraffin blocks in ARMS contained necrotic areas and had limited viable tumor cells with moderate WT1 staining. This may be due to the effect of chemo- or radio-therapy before the surgery. Moreover, the calculation methods used to assess WT1 expression were different across studies and it may also be attributable to the different result. A case report by Ohta et al[22] showed that patient with metastatic ARMS who received weekly intradermal injection with WT1 peptide showed disappearance of metastatic bone lesion after 3 months and the patient had been free of disease for 22 months. Immunotherapy with WT1 peptide can induce WT1-specific cytotoxic T-lymphocytes, that can be detected in high proportion in patient's peripheral blood.[22] Both ERMS and ARMS, which had high WT1 expression as shown in our study, may have promising treatment with WT1 immunotherapy.

In this study, no association was showed between WT1 expression in both ERMS and ARMS with all prognostic factors, including age, tumor size, and tumor location (Table 3). Most of both ERMS and ARMS cases have a tumor size of >5 cm. That tumor size showed higher chance for a lymph node metastasis.[7,19] Kim et al[18] also found that in soft tissue sarcomas, only high tumor grade and advance tumor stage associated with higher WT1 expression, but not for age, tumor size, and tumor location. Tumor grade represents the histology type and tumor stage represents component of tumor size, lymph node involvement, and metastasis tumor. Sotobori et al[20] also demonstrated sex, age, tumor location, tumor size, histopathology grading, and distant metastases at the time of diagnosis were not associated with WT1 mRNA expression.

In this study, ARMS was more common in male but not for ERMS. The data in United States from 1975 to 2005 showed that ERMS was more common in males, with a ratio of 1.5:1, but ARMS cases were similar among females and males.[21] It is reported that 59% of RMS cases occurred in men.[21] Other literatures revealed a bimodal age distribution in ERMS, with a first peak incidence between 0 and 5 years of age and a second peak between the age of 12 and 17 years.[2,6] In this study, most of the RMS patients were younger than 20 years of age when firstly diagnosed. Ognjanovic et al[4] reported that RMS primarily occurs at the age of 0–9 years, followed age 10–19 years, and relatively rare after the age of 20 years. Our study also found that age was not associated with intensity of WT1 staining in ERMS and ARMS. Other studies also found no association between age and WT1 expression.[18,20]

This study showed that RMS was more common in favorable locations. ERMS cases had more favorable locations than ARMS. Van Gaal et al[7] found ERMS was significantly more common in a favorable location, had less lymph node involvement or metastasis, and tended to have a lower stage at diagnosis when compared with ARMS.

There were some limitations of this study that the other RMS prognostic factors were not analyzed in the study and limited sample for ARMS. Further studies about the correlation of WT1 expression and prognostic factors in sarcomas need to be established. In conclusion, WT1 expression was higher in ERMS cases than in alveolar RMS cases, which the expressions were similar in different age, tumor size, and location groups.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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REFERENCES

1. Goldblum JR, Folpe AL, Weiss SW, editors. Enzinger and Weiss's soft tissue tumors. 6th ed. Philadelphia: Elsevier Saunders; 2014. p. 601–34.
2. Parham DM, Barr FG. Embryonal rhabdomyosarcoma. In: Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, editors. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013. p. 127–9.
3. Egas-Bejar D, Huh WW. Rhabdomyosarcoma in adolescent and

young adult patients: current perspectives. Adolesc Health Med Ther. 2014;5:115–25.
4. Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975-2005. Cancer. 2009;115(18):4218–26.
5. Gurney JG, Young JL, Roffers SD, Smith MA, Bunin GR. Soft tissue sarcomas. In: Ries LA, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, editors. Cancer incidence and survival among children and adolescents. United States SEER Program 1975-1995, National Cancer Institute, SEER Program. NIH Pub. No. 99-4649. Bethesda; 1999. p. 111–24.
6. Parham DM, Barr FG. Alveolar rhabdomyosarcoma. In: Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, editors. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013. p. 130–2.
7. Van Gaal JC, De Bont ES, Kaal SE, Versleijen-Jonkers Y, van de Graaf WT. Building the bridge between rhabdomyosarcoma in children, adolescents and young adults: the road ahead. Crit Rev Oncol Hematol. 2012;82(3):259–79.
8. Komdeur R, Klunder J, van der Graaf WT, van den Berg E, de Bont ES, Hoekstra HJ, et al. Multidrug resistance proteins in rhabdomyosarcomas: comparison between children and adults. Cancer. 2003;97(8):1999–2005.
9. Ghosn M, El Rassy E, Kourie HL. Immunotherapies in sarcoma: updates and future perspectives. World J Clin Oncol. 2017;8(2):145–50.
10. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323–37.
11. Van Driessche A, Berneman ZN, Van Tendeloo VF. Active specific immunotherapy targeting the Wilm's tumor protein 1 (WT1) for patients with hematological malignancies and solid tumors: lessons from early clinical trials. Oncologist. 2012;17(2):250–9.
12. Parenti R, Perris R, Vecchio GM, Salvatorelli L, Torrisi A, Gravina L, et al. Immunohistochemical expression of Wilm's tumor protein (WT1) in developing human epithelial and mesenchymal tissues. Acta Histochem. 2013;115(1):70–5.
13. Oue T, Uehara S, Yamanaka H, Takama Y, Oji Y, Fukuzawa M. Expression of Wilms tumor 1 gene in a variety of pediatric tumors. J Pediatr Surg. 2011;46(12):2233–8.
14. Salvatorelli L, Parenti R, Leone G, Musumeci G, Vasquez E, Magro G. Wilms tumor 1 (WT1) protein: diagnostic utility in pediatric tumors. Acta Histochem. 2015;117(4–5):367–78.
15. Magro G, Salvatorelli L, Puzzo L, Musumeci G, Bisceglia M, Parenti R. Oncofetal expression of Wilm's tumor 1 (WT1) protein in human fetal, adult and neoplastic skeletal muscle tissues. Acta Histochem. 2015;117(4–5):492–504.
16. Nakatsuka SI, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, et al. Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol. 2006;19(6):804–14.
17. Carpentieri DF, Nichols K, Chou PM, Matthews M, Pawel B, Huff D. The expression of WT1 in the differentiation of rhabdomyosarcoma from other pediatric small round blue cells tumors. Mod Pathol. 2002;15(10):1080–6.
18. Kim A, Park EY, Kim K, Lee JH, Shin DH, Kim JY, et al. Prognostic significance of WT1 expression in soft tissue sarcoma. World J Surg Oncol. 2014;12:214.
19. Réguerre Y, Martelli H, Rey A, Rogers T, Gaze M, Ben Arush MW, et al. Local therapy is critical in localised pelvic rhabdomyosarcoma: experience of the International Society of Pediatric Oncology Malignant Mesenchymal Tumor (SIOP-MMT) committee. Eur J Cancer. 2012;48(13):2020–47.
20. Sotobori T, Ueda T, Oji Y, Naka N, Araki N, Myoui A, et al. Prognostic significance of Wilms tumor gene (WT1) mRNA expression in soft tissue sarcoma. Cancer. 2006;106(10):2233–40.
21. Sultan I, Qaddoumi I, Yaser S, Rodriguez-Galindo C, Ferrari A. Comparing adult and pediatric rhabdomyosarcoma in the surveillance, epidemiology and end results program, 1973 to 2005: an analysis of 2,600 patients. J Clin Oncol. 2009;27(20):3391–7.
22. Ohta H, Hashii Y, Yoneda A, Takizawa S, Kusuki S, Tokimasa S, et al. WT (Wilms Tumor 1) Peptide Immunotherapy for childhood rhabdomyosarcoma: a case report. Pediatr Hematol Oncol. 2009;26(1):74–83.