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Rhabdomyosarcoma of the Head and Neck: A Clinicopathological and Immunohistochemical Analysis of 29 Cases

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Rhabdomyosarcoma is a malignant tumor occurring more frequently in the childhood. The purpose of this study was to analyze the clinicopathological and immunohistochemical features of rhabdomyosarcomas of the head and neck (RHNs). Twenty nine patients treated in a single institution were selected. The histological slides were reviewed and the tumors were classified. The immunohistochemical reactions were performed using antibodies against vimentin, desmin, myogenin, MyoD1, AE1/AE3, p53, PCNA, Ki67, C-erbB2, FAS and CDK4. The mean age was 14.3 years. The nonparameningeal site was affected in 16 cases (55.2%). Eleven cases (37.9%) affected parameningeal sites and 2 cases the orbit. The p53 was positive in 4 cases (13.8%), CDK4 in 10 cases (34.5%), C-erbB2 in 19 cases (70.4%), FAS in 9 cases (31%), PCNA in 28 cases (96.5%) and Ki67 in 16 cases (55.2%). The overall survival was 28.7% in 5 and 10 years, and p53 expression may be related with poor prognosis.

Key Words: rhabdomyosarcoma, head neck, oral, immunohistochemistry, prognostic factors.

INTRODUCTION

Rhabdomyosarcoma (RMS) is a malignant tumor of skeletal muscle origin, accounting for 5-10% of childhood cancers and for more than 50% of pediatric soft tissue sarcomas (1). About 35% of RMSs are localized in the head and neck region and the main sites of origin for RMSs of the head and neck are: orbit, middle ear, nasal cavity, paranasal sinuses, nasopharynx and infratemporal fossa. According to the site of origin, the RMS of the head and neck can be divided in 3 subtypes: parameningeal, nonparameningeal and orbit. Parameningeal sites are those adjacent to meninges as nasal cavity, paranasal sinuses, nasopharynx, middle ear/mastoid, parapharyngeal space, infratemporal fossa and pterygopalatine fossa. Oral cavity, oropharynx, face, cheek, parotid region and soft tissue of the neck are considered nonparameningeal sites (2).

Histologically, the RMSs can be divided in embryonal, alveolar and pleomorphic. The embryonal type presents the subtypes: classic, spindle cell and botryoid. The embryonal RMS is composed of several cell types and the mesenchymal cells tend to differentiate into cross-striated muscle cells. It is generally a moderately cellular tumor with loose myxoid stroma. The cell nuclei of embryonal RMS are smaller than those of an alveolar RMS and the nucleoli are difficult to visualize (3). The immunohistochemical positivity for vimentin, muscle-specific actin, desmin and mioglobin has been used to distinguish RMS of other soft tissue sarcomas with similar histopathological patterns. Myogenin and MyoD1, myogenic transcriptional regulators, seem to be more specific to identify myogenic cell line (4).

The classification of RMSs is based on the

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tumor, nodes and metastases (TNM) staging system and the classification proposed by the International Rhabdomyosarcoma Study Group (IRSG). The TNM classification uses not only clinical features, but also the histological grade (UICC). The IRSG classification is based on clinical aspects including local invasion, the possibility of total surgical resection, and the presence of regional or distant metastasis (5). The treatment of RMSs of the head and neck has still some controversy. In the past, the prognosis of RMSs of the head and neck was very poor. However, with the introduction of multidrug chemotherapy, the survival in this group of patients has improved. Afterwards better survival was obtained with combination of multidrug chemotherapy, external beam radiotherapy, and/or surgery (6). In 1998, Schouwenburg et al. (7) published the AMORE protocol, which consisted of ablative surgery, brachytherapy and reconstructive surgery for residual or recurrent disease after multidrug chemotherapy, with good results.

Alterations in cell cycle gene control have been identified in several tumors and may play a role in the development of RMSs. Mutations of the tumor suppressor gene P53 is the most frequent genetic alteration in human cancer. CDK4 (cyclin dependent kinase 4) has been demonstrated to be high-expressed in a great variety of malignancies, including RMSs (8). C-erbB2 is a proto-oncogene frequently found in breast cancer and Ricci et al. (9) reported positivity for C-erbB2 in 3 out of 5 RMS. Fatty acid synthase (FAS) is an enzyme responsible for the endogenous synthesis of saturated long-chain fatty acids from the precursors acetyl-CoA and malonyl-CoA. It has been demonstrated that FAS expression is up-regulated in a variety of human malignancies, including soft tissue sarcomas (10).

This study evaluated the clinical, histopathological and immunohistochemical features of RMSs of the head and neck and their relationship with the prognosis.

MATERIAL AND METHODS

All cases of RMSs of the head and neck treated in the Department of Head and Neck Surgery of A.C. Camargo Hospital, São Paulo, SP, Brazil, between 1954 and 2002 were included in this series. The clinical and demographic data were obtained from the medical charts. The diagnosis of RMS was confirmed by histological and immunohistochemical analysis. The primary antibodies used to confirm the diagnosis were: anti-desmin (D33; Serotec, Oxford, UK: 1/1000), anti-vimentin (Vim 3B4; Dako A/S, Denmark: 1/400), anti-MyoD1 (5.8A; Dako A/S, Denmark: 1/50), anti-myogenin (LO26; Novocastra, Newcastle: 1/40), anti-AE1/AE3 (AE1 & AE3; Dako A/S, Denmark: 1/500). The cases confirmed for the diagnosis of RMS were histologically classified as embryonal, alveolar or pleomorphic. The embryonal subtype was sub-classified as: classic, botryoid and spindle-cell. To evaluate the prognosis importance, other antibodies were tested: anti-p53 (DO-7; Dako A/S, Denmark: 1/200), anti-CDK-4 (sc-260-G; Santa Cruz Biotechnology, Santa Cruz: 1/400), anti-Ki67 (MIB-1; Dako A/S, Denmark: 1/200), anti-PCNA (PC10; Dako A/S, Denmark: 1/16000), anti-FAS (23; BD Biociences: 1/2000), anti-C-erbB2 (p185 HER2; Dako A/S, Denmark: 1/200). The positivity was classified as negative (0-5% of the tumor cells), weak (6-25%), moderate (26-50%) and strong (more than 50%).

The tumors were classified according to the IRSG and TNM classifications and correlation of frequency among the clinical, histological and immunohistochemical features were performed with Fisher´s exact test. The overall and free-disease survival were calculated with Kaplan-Meier method and survival curves were compared using the log-rank statistic test.

RESULTS

Twenty-nine cases were confirmed for the diagnosis of RMS. The mean age of the patients was 14.3 years, ranging from 1.5 months to 72 years. Most cases (51.7%) were found in the first decade of live. There was a male predominance (58.6% of the cases). The nonparameningeal site was predominant with 16 cases (55.2%), and 10 of them occurred in the oral cavity (4 in the buccal mucosa, 2 in the tongue, 2 in the palate, 1 in the upper lip and 1 in the floor of the mouth). The parameningeal sites were affected in 11 cases (37.9%), the nasal cavity being the most common (5 cases). The orbit was involved only in 2 cases (6.9%). The mean time of the signals and symptoms from the onset to the diagnosis was 6.6 months, ranging from 20 days to 72 months, and the most common complaint was swelling. Pain was found in 13 cases (44.8%), and the mean size of the tumors was 5.6 cm, ranging from 1.5 cm to 10 cm.

Almost all cases were positive for vimentin (28 cases - 96.5%) and desmin (27 cases - 93.1%). Myogenin was positive in 27 cases (93.1%) and MyoD1 in 10 cases

(34.5%). The cases negative for vimentin or desmin were positive for myogenin or MyoD1, confirming the diagnosis of RMS. In the histological analysis the embryonal RMS was the most frequent with 18 cases (62.1%), being 15 of them sub-classified as classic, 2 as botryoid and 1 as spindle-cell. Ten cases (34.5%) were classified as alveolar, being 8 as classic and 2 as solid. Only one case was classified as pleomorphic (3.4%).

The immunohistochemical analysis revealed positivity for p53 in 4 cases (13.8%) and all of them considered weakly positive. The CDK4 was evaluated for nuclear and citoplasmic positivity. Nuclear positivity was found in 10 cases (34.5%), 3 strong, 1 moderate and 6 weak. C-erbB2 was positive in 19 cases (70.4%), 13 strong, 4 moderate and 2 weak. FAS protein was positive in 9 cases (31%), 4 strong, 4 moderate and 1 weak. PCNA was positive in 28 cases (96.5%), 23 strong, 1 moderate and 4 weak. Ki67 was positive in 16 cases (55.2%), 3 strong, 4 moderate and 9 weak.

According to the TNM classification, 18 cases (62.1%) were classified as stage I, 6 cases (20.7%) stage II, 3 cases (10.3%) stage III and 2 cases (6.9%) stage IV. And according to the clinical group (IRSG), 18 cases (66.7%) were classified as IIIA, 3 cases (11.1%) as IIA, 2 cases (7.4%) as IV and IA, IB, IIB and IIIB had one case each.

The association of chemotherapy and radiotherapy was the treatment of choice in 17 cases (58.6%). The surgical resection was performed in 8 cases, being one case exclusively surgery, 2 surgery associated with radiotherapy, 2 surgery with chemotherapy and in 3 cases surgery plus radiotherapy and chemotherapy. Chemotherapy alone was the treatment of choice in 4 cases. Radiotherapy was performed with a mean local dose of 43.4 Gy, ranging from 20 to 70 Gy. Local recurrence occurred in 13 cases (44.8%), regional metastasis in 8 cases (27.6%) and distant metastasis in 6 cases (20.7%). In 4 patients was observed local recurrence and regional metastasis, in one patient local recurrence with distant metastasis, in 3 patients local recurrence, regional and distant metastasis, and in one patient regional and distant metastasis.

The correlation of frequency among all the clinical, histological and immunohistochemical features did not show any statistical difference. The overall survival was 28.7% both in 5 years and 10 years and the disease-free survival probability was 24.7% both in 5 and 10 years (Fig. 1). In the Kaplan-Meyer actuarial survival rates calculated according to the clinical, histological and immunohistochemical findings, only the p53 could be associated to the prognosis, where the positive cases presented a worse survival comparing to the negative (p=0.09) (Table 1).

DISCUSSION

The RMS affects more commonly children, being considered the most common soft-tissue sarcoma of childhood, with an annual incidence of 4 to 7 million children 15 years of age or younger and a male predominance. Pappo (1) described 67% of the patients with less than 14 years old. Maurer et al. (11) in the intergroup RMS study reported that 87% of the patients had 14 years or less among 999 patients, and they also found a male predilection with 73% of the cases. Simon et al. (12) studying 49 head and neck RMS found a mean age of 6.2 years and a male predominance (63%). In our series the mean age was 14.3 years with 51.7% in the first decade and a male predominance with 58.6% of the cases.

In head and neck region the RMSs are divided in parameningeal (adjacent to the meninges), nonparameningeal and orbit. Simon et al. (12) found most of the tumors (51%) in parameningeal sites; nonparameningeal and orbit were affected in the same proportion (24.5%). In the present study, there was a predominance in the nonparameningeal region, with 16 cases (55.2%). Ten out these 16 nonparameningeal tumors affected the oral cavity: 4 in the buccal mucosa, 2 in the tongue, 2 in the palate, 1 in the lip and 1 in the floor of the mouth.

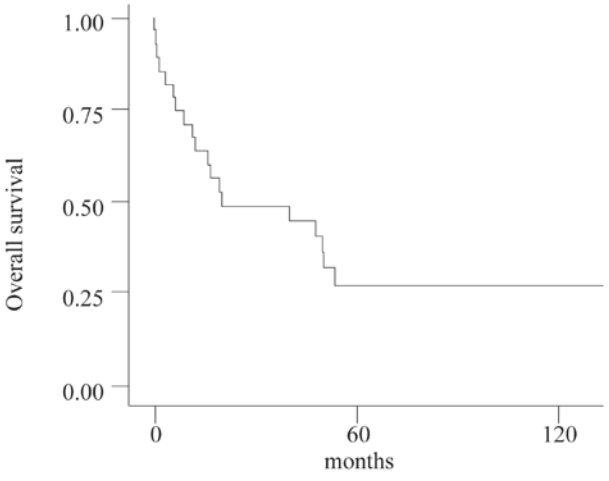

Figure 1. Overall survival in 60 and 120 months.

Maurer et al. (6) found 79% of the tumors with less than 5 cm. Cavazzana et al. (13) studying spindle-cell embryonal RMSs reported a mean size of 4.4 cm. Simon et al. (12) reported that 57% of the cases the tumor size were less than 5 cm and the mean period from the onset to the diagnosis was 7 months. Al-Khateeb and Bataineh (14) studying RMS from the oral and maxillofacial region reported that the persistent swelling of with duration of about 6 weeks was the main complaint of the patients. Pain was associated in about 50% of the cases. In our series we found a larger mean size of the tumors (5.6 cm) with most of them between 5 and 10 cm, and the time of complaint was about 6 months.

Table 1. Overall survival of 29 patients with RMS of the head and neck according to the clinical, histological and immunohistochemical variables.

	Overall survival (%)		
	5 years	10 years	p-value
Age			
≤ 10 years	24.3	24.3	0.7139
≥ 10 years	32.3	32.3	
Location			
Nonparameningeal	41.7	41.7	0.4969
Parameningeal	20.4	20.4	
Orbit	0.0 (≤2 yrs)	----	
Histological type			
Embryonal	23.8	23.8	NA
Alveolar	0.0 (≤1 yr)	----	
Pleomorphic	38.9	38.9	
Immunohistochemistry			
P53			
Positive	0.0 (≤4 yrs)	----	0.0922
Negative	33.8	33.8	
CDK4 (nuclear)			
Positive	35.5	35.5	0.4769
Negative	24.8	24.8	
CDK4 (citoplasmatic)			
Positive	31.6	31.6	0.8618
Negative	24.1	24.1	
C-erbB2			
Positive	27.7	27.7	0.5110
Negative	26.4	26.4	
FAS			
Positive	22.2	22.2	0.3930
Negative	32.9	32.9	
Ki67			
Positive	24.9	24.9	0.6437
Negative	33.3	33.3	

The most common histological type of RMS is the embryonal, representing 50% to 70% of all cases (11-14). The alveolar subtype is the second most common, accounting for approximately 20% to 30% of the cases (11,12,14). The pleomorphic subtype is the rarest and comprises about 5% of the cases (6,11). When RMS affects adults the pleomorphic subtype is the most common. In our series we classified only one case as pleomorphic RMS, representing 3.4% of the cases.

The differential diagnosis of RMS from other poorly differentiated tumors can be difficult or sometimes impossible only on routine HE staining. The use of immunohistochemistry for vimentin and desmin may only suggest the skeletal-muscle differentiation. The expression of markers specific for rhabdomyoblastic differentiation is particularly useful for the diagnosis of RMS. Myogenin and MyoD1 are myogenic transcriptional regulators. They are expressed earlier in skeletal muscle differentiation than desmin, muscle-specific actin, myoglobin and myosin. Desmin and myogenin are positive in about 90% of the RMSs. It is described that the sensitivity for MyoD1 in RMS ranges from 71% to 91% (15). However, the MyoD1 staining may be limited in archival formalin-fixed, paraffin-embedded samples. In our series myogenin was positive in 27 cases (93.1%) and MyoD1 in 10 cases (34.5%). The diagnosis of RMS was confirmed only if at least one of these proteins were positive.

p53 has been studied in several tumors and its immupositivity in RMSs varies in different series ranging from 5% to 44% of the cases (16,17). CDK4 (cyclin-dependent kinase 4) is another cell cycle control gene that is reported to be positive in about 80% of the alveolar RMSs. Analyzing head and neck ostesarcomas, Takahama et al. (18) reported immunopositivity for CDK4 in 84% of the cases and for p53 in 52%. C-erbB2 expression is reported to be found in 60% of the RMSs (9). Takahiro et al. (10) studying the expression of FAS in soft tissue sarcomas reported a positivity of 31% of the cases. However, none of the studied cases of RMS was positive. In the present study, p53 was positive in 13.8%, CDK4 in 34.5%, C-erbB2 in 70.4% and FAS in 31%.

Simon et al. (12), studying 49 RMSs of the head and neck found 24 cases (62%) in the group III. Al-Katheeb and Bataineh (14) also classified most of the cases (55%) in the group III. These findings are in agreement with our results were 74% of the patients

were classified in the group III.

Local control is the main objective on the treatment for head and neck RMS. Multimodality treatment protocols, including surgery, radiotherapy and chemotherapy has improved the outcome in the past decades. In 1998, Schowenburg et al. (7) described the AMORE protocol for patients with RMS of the head and neck which consisted on ablative surgery, moulage technique with afterloading brachytherapy and reconstructive surgery directed to the residual tumor after multiagent chemotherapy. In this protocol, the brachytherapy was introduced to reduce the dose of radiation in health tissue, avoiding the long-term sequelae, especially when applied in young children (19). Our patients received different types of treatment according to the year of the treatment, considering the interval of almost 50 years that we included the cases.

Maurer et al. (11) analyzing head and neck RMSs reported overall survival of 92% in 5 years for tumors located in the orbit. In nonparameningeal tumors, this number decreased to 81% and in parameningeal to 69%. Simon et al. (12) found a lower survival with 60% in 5 years, including all the sites of head and neck. The prognosis of patients with parameningeal RMS is considered the worst, in spite of the combination of treatment modalities, with a survival rate of 28% after 5 years (7). Simon et al. (12) reported that improvement in outcome was obtained with multimodality treatment and both tumor size and age of the patient were found to influence survival. The present study revealed that overall survival was 28.7% both in 5 years and 10 years and the worse survival was found in the parameningeal sites with 20.4% comparing to the nonparameningeal with 41.7%. However this difference was not significant on the statistical analysis.

Studying 42 cases of RMS, Ayan et al. (16) reported that nuclear p53 expression was associated with disease progression or recurrence and with a worse event free survival. On the other hand, Leuchner et al. (20), studying 150 cases of RMS did not find correlation of p53 expression with the prognosis. In our series, the p53 expression seems to be related with a worse prognosis, however this difference was of marginal statistical significance (p=0.09).

In summary, 29 patients were diagnosed and treated for RMS of the head and neck over a period of 48 years in the A. C. Camargo Hospital. Most of them were children and received multimodality therapy, including surgery, chemotherapy and radiotherapy. The only variable that seems to be related with the prognosis was the immunohistochemical positivity for p53.

RESUMO

Rabdomiossarcoma é um tumor maligno que ocorre mais frequentemente na infância. O objetivo deste estudo foi analisar as características clinicopatológicas e imunohistoquímicas dos rabdosiossarcomas de cabeça e pescoço. Vinte e nove pacientes tratados em uma única instituição foram selecionados. As lâminas histológicas foram revisadas e os tumores foram classificados. As reações imunohistoquímicas foram realizadas usando anticorpos contra vimentina, desmina, miogenina, MyoD1, AE1/AE3, p53, PCNA, Ki67, C-erbB2, FAS e CDK4. A idade média dos pacientes foi de 14,3 anos. Localização não-parameningeal foi o sítio mais afetado correspondendo a 16 casos (55,2%). Onze casos (37,9%) afetaram sítios parameningeais e em 2 casos a órbita. p53 foi positivo em 4 casos (13,8%), CDK4 em 10 casos (34,5%), C-erbB2 em 19 casos (70,4%), FAS em 9 casos (31%), PCNA em 28 casos (96,5%) e Ki67 em 16 casos (55,2%). A sobrevida global foi 28,7% em 5 e 10 anos, e a expressão de p53 pode estar relacionado ao pior prognóstico.

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