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Clinicopathologic features of 300 rhabdomyosarcomas with emphasis upon differential expression of skeletal muscle specific markers in the various subtypes: A single institutional experience

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ABSTRACT

The present study was aimed at evaluating clinicopathologic and immunohistochemical (IHC) features of 300 rhabdomyosarcomas (RMSs), including differential IHC expression and prognostic value of myogenin and MyoD1 across various subtypes of RMSs.

IHC expression of myogenin and MyoD1 was graded on the basis of percentage of tumor cells displaying positive intranuclear immunostaining i.e. grade 1 (1–25%); grade 2 (26–50%); grade 3 (51–76%) and grade 4 (76–100%).Clinical follow-up was available in 238 (79.3%) patients. Various clinicopathologic parameters were correlated with 3-year disease free survival (DFS) and overall survival (OS).

There were 140 cases (46.7%) of alveolar RMS (ARMS), 90 of embryonal RMS (ERMS) (30%), 61 (20.3%) of spindle cell/sclerosing RMS and 9 cases (3%) of pleomorphic RMS. Most cases, barring pleomorphic RMSs, occurred in the first two decades (228 cases) (76%), frequently in males, in the head and neck region (126) (42%).

By immunohistochemistry, desmin was positive in 292/299 (97.6%) tumors; myogenin in 238/267 (89.1%) and MyoD1 in 192/266 (72.2%) tumors. High myogenin expression (in ≥51% positive tumor cells) was significantly associated with ARMSs (95/121, 78.5%), as compared to other subtypes (48/117, 41%) (p value < 0.001). High MyoD1 expression (≥51% tumor cells) was seen in more cases of pure sclerosing, combined with spindle cell/sclerosing RMSs (10/10, 100%), as compared to the other subtypes (91/141, 67.4%) ($p = 0.032$).

There was no significant difference between high myogenin expression and clinical outcomes. Patients without metastasis and harbouring tumors, measuring ≤5 cm showed a significant increase in OS, with p values = 0.01 and < 0.001, respectively.

ARMS was the most frequent subtype. There was a significant association between high myogenin expression and ARMSs and high MyoD1 expression and spindle cell/sclerosing RMSs. High myogenin expression did not correlate with clinical outcomes. Patients with smaller sized tumors and without metastasis had significantly better clinical outcomes.

1. Introduction

According to the recent World Health Organization (WHO) classification, various subtypes of rhabdomyosarcoma (RMS) include embryonal (ERMS), alveolar, (ARMS), pleomorphic RMS, along with the newly recognized, spindle cell/sclerosing subtype [1-4].

Traditionally, alveolar and pleomorphic RMSs have been found to be relatively more aggressive subtypes of a RMS [1]. Other, relatively aggressive prognostic variables in cases of RMS include age of the patient (adults than children), T-size (exceeding 5 cm), metastasis at presentation and a higher TNM stage [5-8]. Within cases of ARMS, the ones associated with a specific translocation, t(2; 13) (*PAX3-FKHR*) are

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known to be associated with relatively more aggressive clinical outcomes than those harbouring t(1; 13) (*PAX7-FKHR*) [9].

Earlier, few studies have shown diagnostic and prognostic value of skeletal muscle specific markers, such as myogenin in cases of ARMS [10-13]. Recent studies have shown association between MyoD1 immunoexpression and sclerosing RMSs [14,15] Furthermore, *MyoD1* mutations have been reported exclusively in certain cases of spindle cell/sclerosing rhabdomyosarcomas, associated with relatively aggressive clinical outcomes [16-19].

The present study was aimed at evaluating clinicopathologic and IHC features of 300 cases of RMS, including differential expression of skeletal muscle specific markers, including their prognostic significance.

2. Material and methods

This study was approved by the Institutional Ethics Committee (IEC) and included retrospective analysis of 300 diagnosed cases of RMS, including patients registered at our Institution from the year 2005 to 2013 (9 years), along with 3 cases with available clinical details, registered in the last 2 months of the year 2004 and a single case enrolled in the first month of 2014. The study was censored at a total of 300 cases.

The cases were retrieved from the Hospital "search engine". Clinical details were obtained from the electronic medical records (EMR) and case files from the medical records department of our Institution.

Clinical parameters studied were age of the patient, gender, tumor (T) size and site of the primary tumor, which was further sub classified as favorable or unfavorable. Favorable sites included orbit, head and neck region, excluding parameningeal area, and genitourinary region, excluding urinary bladder and prostate. The other sites were considered as unfavorable. Various other parameters were evaluated, such as status of metastasis, (combined regional lymph nodal and distant metastasis), status of distant metastasis, site (s) of metastasis, treatment modality (surgery, chemotherapy, radiotherapy), type of chemotherapy protocol and recurrence (relapse).

The diagnostic material in 300 cases was in the form of biopsy specimens (216 cases, 72%), either as formalin preserved tissues or in the form of paraffin block(s) and in the form of excision specimens (84 cases, 28%), either as formalin preserved tissues or in the form of paraffin blocks. Among 216 cases with biopsy specimens, 12 cases had post treatment specimens and 4 cases had post treatment biopsies.

Conventional hematoxylin and eosin (H&E) stained slides were available for a review, by B.R with C.G, in all 300 cases. Criteria for the diagnosis of RMS, including its various subtypes were based on the recent WHO classification of tumors of soft tissues and bone [1-4].

Apart from cases of ERMS, ARMS (including few cases with mixed features of ARMS and ERMS) and pleomorphic RMS; cases of spindle cell/sclerosing RMS were designated as 'pure' spindle cell RMS (having predominant spindle cell features in > 75% tumor), 'pure' sclerosing RMS (having predominant sclerosing features in > 75% tumor) and spindle cell/sclerosing RMS (having both the features, nearly in equal proportions until 25% more of either type of tumor components). These included previously reported cases of spindle cell/sclerosing RMSs [15,19].

Immunohistochemistry was carried out formalin-fixed, paraffin-embedded tissue, using an avidin–biotin peroxidase complex system. During review of immunostained microsections, only intranuclear staining pattern of myogenin and MyoD1 within the tumor cells was considered as positive. Furthermore, IHC expression of myogenin and MyoD1 was graded: Grade 1 (1–25%) (low positive); grade 2 (26–50%) (moderately positive); grade 3 (51–76%) (highly positive) and grade 4 (76–100%) (very highly positive).

Immunoexpression of myogenin and MyoD1 were evaluated in various subtypes of RMS, including 'pure' sclerosing RMS and spindle cell/sclerosing RMS and was further compared with the expression of the same in the other subtypes (excluding the cases of 'pure' spindle cell RMS). Expression of myogenin and MyoD1 was also evaluated in the cases of spindle cell RMS and compared with the other subtypes (excluding the cases of sclerosing RMS and spindle cell/sclerosing RMS).

Results of RT-PCR test, for *PAX3-FKHR* and *PAX7-FKHR* gene fusions, available in 20 cases, wherever performed, were noted.

For calculating clinical outcomes, date of diagnosis, date of initiating treatment, date of relapse/recurrences and date of last follow-up, including death, wherever available, were recorded. Date of the diagnosis was considered as the initial point during survival analysis.

2.1. Statistical analysis

Descriptive statistics were employed using SPSS version 20, including log rank (Mantel-Cox) test and Pearson Chi-Square test. p value < 0.05 was considered as statistically significant.

Survival analysis was done using Kaplan Meier test. Overall survival (OS) was defined as the duration between the initial point (date of diagnosis) till the date of death. Disease free survival (DFS) was defined as the duration from the initial point till the date of first occurrence of relapse/recurrence and/or metastasis. Patients not experiencing an event at the date of their last follow-up were censored. Cases with a follow-up duration of < 3 months were excluded from the survival analysis.

Clinical outcomes/follow-up details were available in 242 (80.6%) patients. In 242 (80.6%) patients, OS with follow-up exceeding 3 months could be evaluated, whereas in 75 patients (25%), DFS with follow-up exceeding 3 months could be evaluated, considering only those patients were included, who developed events after diagnosis.

Various clinicopathologic parameters were correlated with disease free survival (DFS) and overall survival (OS).

Furthermore, the distribution pattern on censored observation was tested to check the sensitivity of the survival analysis. The intention was to observe that any parameters are affected by more number of censored observations or not. The distribution pattern of duration of follow-up among censored cases was tested through an independent t-test. The parameters, such as age ($\leq$15 and > 15 years), sites (head and neck and extremities; trunk and extremities), histopathologic subtype (ERMS and ARMS; ARMS and Spindle cell/sclerosing) and tumor size ($\leq$5 and > 5 cm), were analyzed to check the difference in censored cases.

3. Results

Age of the patients ranged from newborn to 78 years, with an average and median age of 14.7 years and 11 years, respectively. Out of 300 patients, 203 (67.7%) were males (M) and 97 (32.3%) were females (F), with M: F ratio of 2.1:1.

The tumor locations included various soft tissue sites in 170 cases (56.7%) and head and neck region in 126 cases (42.0%). The soft tissue sites included extremities as the most common location in 59 cases (19.7% of all cases) and other sites, including genitourinary region, retroperitoneal area, intra-thoracic, intra-abdominal, trunk, pelvic region, paravertebral area, perineal and perianal area. The primary location of the tumor was not known in 4 cases (referral cases) (1.3%). The tumors arising from the favorable sites were 101/300 (33.7%) and from the unfavorable sites were 195/300 (65%).

Most cases, including various subtypes, except pleomorphic RMSs, occurred in the first two decades of life (228 cases) (76%), frequently in males and in the head and neck region (126) (42%), followed by extremities (59) (19.7%).

T-size (known in 182 cases, 60.6%) was $\leq$5 cm in 66 cases (36.3%) and exceeding 5 cm in 116 cases (63.7%).

Clinical status regarding metastasis was known in 250 cases (83.3%). Out of 250 cases, metastatic lesions were present in 134 cases (53.6%). The common sites of metastases included lymph nodes (95