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Original Article

Pathology Section

Clinicopathological Evaluation of Rhabdomyosarcoma with Emphasis on Myogenin Expression and *FOXO1* Gene Fusion in Various Morphological Subtypes: A Cohort Study

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ABSTRACT

Introduction: Rhabdomyosarcoma (RMS) is a prevalent paediatric soft-tissue sarcoma. Among the four histomorphological subtypes of RMS, distinguishing the dense pattern of Embryonal RMS (ERMS) from the solid pattern of Alveolar RMS (ARMS) solely based on morphology is challenging and necessitates ancillary techniques.

Aim: To study the demographics, classify the histomorphological subtypes and reclassify the morphologically overlapping embryonal and ARMS cases into specific subtypes based on the intensity of myogenin expression and the *FOXO1* gene fusion status.

Materials and Methods: This cohort study was conducted at the Kidwai Memorial Institute of Oncology, Bengaluru, Karnataka, India, from January 2019 to December 2022. The study included 71 cases of RMS. Clinical data such as age, gender, tumour site, size and clinical stage, along with histomorphological types, were analysed. A panel of immunomarkers was performed based on morphological differentials, which included desmin, myogenin, MyoD1, CD99, synaptophysin, chromogranin, PanCK, NKX2.2, INI1, CD56, S100, CD34 and SMA. Myogenin expression was scored based on the extent of tumour cell positivity. Interphase Fluorescence In Situ Hybridisation (FISH) analysis was conducted on all ARMS and morphologically unclassified cases using the CytoTest Break Apart Probe for the FOXO1 gene. One hundred tumour cells were analysed and split signals, with or without amplification signals in at least 15 cells, were considered positive.

Results: A male preponderance was noted (43/71, 60.6%), with a higher incidence among children under 14 years (42/71, 59.2%). ERMS was the most common histological subtype (26/71, 36.6%), followed by ARMS (13/71, 18.3%) and Spindle Cell RMS (SCRMS) (7 cases, 9.9%). The head and neck regions were frequently involved (24 cases, 33%). Twenty-three cases of unclassified RMS were reclassified into ARMS (fusion-positive) (43.4%, n=10) and reclassified ERMS (fusion-negative) (34.7%, n=8). Notably, the correlation between myogenin expression and *FOXO1* fusion showed that 94.7% of fusion-positive cases exhibited 4+ myogenin expression (p-value <0.001). Overall, ARMS had the worst Overall Survival (OS) rate (26.1%). The reclassified ERMS and the classic ERMS cases showed almost similar survival rates (62.5% vs 64.2%, respectively) (p-value=0.025).

Conclusion: The study highlights that the myogenin immunomarker is useful in differentiating between ERMS and ARMS in resource-constrained settings and emphasises the need for fusion testing in ARMS and unclassified RMS cases for accurate risk assessment and tailored treatment strategies.

Keywords: Fluorescence in situ hybridisation, Rhabdomyosarcoma subtypes, Soft-tissue sarcoma

INTRODUCTION

The fifth edition of the World Health Organisation (WHO) recognises four subtypes of RMS, which include embryonal [1], alveolar [2], spindle cell/sclerosing [3] and pleomorphic RMS [4]. Among these subtypes, the solid pattern of ARMS and the dense pattern of ERMS exhibit morphological overlap, with subtle differences in their cytomorphology. This poses a significant challenge for pathologists when subtyping these tumours, especially in needle core biopsies. However, differentiating between the two subtypes is imperative due to their distinct biological potentials [5]. The primary objective of this study was to analyse the various clinicopathological characteristics of RMS cases and to classify the morphologically overlapping embryonal and alveolar subtypes into specific subtypes based on myogenin expression and *FOXO1* gene fusion. The secondary objective was to study the outcomes of these cases by estimating Overall Survival (OS). Very limited studies have been conducted in the past on the correlation of myogenin expression with histomorphological subtypes [6-9]. However, molecular correlation using *FOXO1* FISH with myogenin expression has not been carried out in previous studies. To the best of our knowledge, following the release of the fifth edition of the WHO classification on soft-tissue and bone tumours, this was the first study from our country to analyse the fusion status and myogenin expression in various RMS subtypes, along with the analysis of their clinical outcomes.

MATERIALS AND METHODS

This was a cohort study conducted by collecting all cases of RMS that were diagnosed at the Kidwai Memorial Institute of Oncology, Bengaluru, Karnataka, India from January 2019 to December 2022. The study received approval from the institutional scientific review board and ethics committee (MEC/202304/PG/PT/06), adhering to the principles of the Helsinki Declaration II. Informed consent from patients was waived, as this was a retrospective study.

Inclusion criteria: All confirmed cases diagnosed in biopsies as well as resections were included in the study.

Exclusion criteria: Other small round cell tumours that tested negative for myogenic markers were excluded from the study. The study population included 71 cases of RMS.

Study Procedure

Histology and Immunohistochemistry (IHC) slides of all the cases were retrieved and reviewed by two oncopathologists. No interobserver variability was observed in cases presenting with classic morphology. However, variability was noted in 4 out of 23 cases that exhibited overlapping morphology between ERMS and ARMS, reinforcing the necessity for molecular studies in such instances.

Cases were categorised into four WHO subtypes based on histomorphology: ERMS, which includes classic, botryoid, anaplastic and spindle cell patterns [1]; ARMS, characterised by the classic alveolar pattern [2]; SCRMS, which includes fibrosarcomatous or sclerosing patterns [3]; and pleomorphic RMS, noted for the presence of highly pleomorphic bizarre cells [4]. In cases where specific patterns or histomorphology were lacking, those with mixed ERMS and ARMS histology, or those with insufficient tissue for subclassification, were designated as RMS unclassifiable (ERMS/ARMS). Head and neck, orbit (excluding parameningeal and paraspinal areas), and genitourinary (excluding bladder and prostate) sites were considered favourable, while other sites were classified as unfavourable.

IHC was performed using the Ventana Benchmark XT with markers such as myogenin (F5D, DBS, CA), MyoD1 (rMYD712, Biogenex, CA), and desmin (D33, DBS, CA). Additional markers, like CD99 (H036-1.1, DBS, CA), NKX2.2 (ZM-14, 1:200, ZETA, CA), synaptophysin (SYP02, DBS, CA), chromogranin A (LK2H10, BioSB, CA), pan-cytokeratin (AE1+AE3, DBS, CA), SMA (1A4, Biogenex, CA), CD56 (123C3.D5, DBS, CA), S100 (4C4.9, DBS, CA), CD34 (QBEND/10, DBS, CA), and INI1 (25, ZETA, CA) were also used where necessary to rule out other small round cell and spindle cell neoplasms. Upon review, the percentage of myogenin expression was scored from 1+ to 4+ {1+ (<10% cells), 2+ (10-50% cells), 3+ (50-90% cells), 4+ (>90% cells)} [6-9]. We also checked for aberrant expression of immunomarkers in all the cases during the review.

Interphase FISH analysis was performed using the CytoTest Break Apart Probe (Rockville, USA) for the *FOXO1* gene at 13q14 on Formalin-Fixed Paraffin-Embedded (FFPE) tissues. The probe comprises a spectrum orange-labelled 520 Kb locus-specific probe flanking the 5' end and a 590 Kb spectrum green-labelled probe flanking the 3' end of the *FOXO1* gene. The FFPE sections were baked at 60°C for 60 minutes, deparaffinised with xylene and cleared with absolute alcohol. They were then pretreated with 0.2N hydrochloric acid for 20 minutes at Room Temperature (RT) and with 1M sodium thiocyanate at 80°C for 35 minutes before being digested with the enzyme protease at 37°C for 10 to 15 minutes. The sections were washed with 2X Sodium Saline Citrate (2SSC) and denatured in a 70% formamide solution. They were dehydrated with graded alcohol.

Ten µL of a solution containing the CytoTest *FOXO1* break apart probe was applied to the target area, which was sealed with rubber cement and kept for denaturation for five minutes at 75°C, followed by hybridisation at 37°C for 16 to 20 hours in a humidified chamber (Euroclone, Pero, Italy). Following hybridisation, the sections were washed in NP40 (Nonidet P-40) and counter-stained with 4,6-diamidino-2-phenylindole (DAPI). The FISH signals were captured and analysed using the Applied Spectral Imaging (ASI) basic workstation, version 7.2.7.34276 (Santa Clara, California). Scoring was performed on 100 non overlapping tumour nuclei. Break-apart signals, with or without amplification signals, were considered positive when at least one signal was observed at a distance apart in a minimum of 15% of cells. Any number of fusion signals (fusion±amplification) without break-apart signals were considered negative. The test was repeated when poor signal intensity and/or high background noise were noted.

Clinical, treatment and follow-up data were collected from medical records and a modified TNM pretreatment staging classification system was followed to stage the tumours [10].

STATISTICAL ANALYSIS

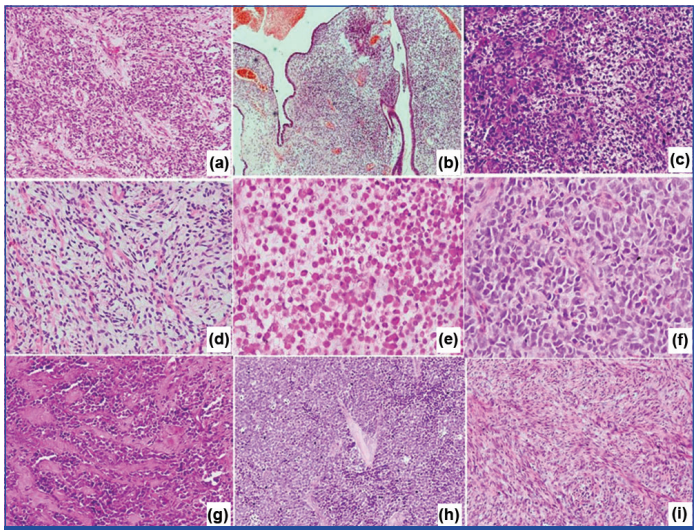
Statistical analysis was conducted using the software "R", with the Pearson Chi-square test and Fisher's exact test used to test the associations between variables. A p-value of <0.05 was considered statistically significant. OS was estimated using Kaplan-Meier analysis. OS was defined as the time from study entry until death or the last follow-up.

RESULTS

A total of 71 cases were studied, comprising 43 children, 20 adolescents, and eight adults. The age range varied from one month to 70 years, with a median age of 13 years and a mean age of 14.7 years. A male preponderance was noted, with a male-to-female ratio of 1.5:1. The cases were diagnosed through needle core biopsies (49 cases, 69%) and resections (22 cases, 31%), and were confirmed by IHC. The head and neck was the most common site involved (24 cases, 33%), with specific locations including the nasal cavity (six cases), parotid gland (two cases), maxilla (two cases), parapharyngeal space (one case), external auditory canal (four cases), neck (four cases), and orbit (five cases). Overall, 32 cases (45%) were seen in favourable sites, while 34 cases (47.8%) were in unfavourable sites. In five cases, the primary site was unknown, and they presented at metastatic sites such as lymph nodes (three cases), lung (one case), and bone (one case).

Histological Subtype

ERMS was the most common histological subtype, comprising 28 cases (42.4%) with classic, botryoid, anaplastic, or spindle cell morphology. ARMS, characterised by a typical alveolar growth pattern, was observed in 13 cases (18.3%), and seven cases (9.8%) of SCRMS, including those with a sclerosing pattern, were observed. Additionally, 23 out of 71 cases (32.3%) presented with scanty tumour tissue composed of small round cells arranged in diffuse sheets or a solid pattern, and these were grouped under the RMS unclassified category after confirmation by IHC. [Table/Fig-1] shows the various histomorphological subtypes of RMS. ERMS was predominantly found in the head and neck, bladder and para-testicular areas, with the majority affecting favourable sites (60.7%). Of the ERMS cases, 64.2% occurred in children, with a median age of eight years. ARMS cases were commonly found in the upper and lower extremities, with the majority affecting unfavourable sites (52.1%). Of the ARMS cases, 69.5% occurred in children, with a median age of 11 years. SCRMS cases showed no age or site predilection. No cases of pleomorphic RMS were reported.



[Table/Fig-1]: Various histomorphological subtypes of RMS (H&E, 10x): a) Classic pattern ERMS; b) Botryoid pattern ERMS; c) Anaplastic pattern ERMS; d) Spindle cell pattern ERMS; e) Post-chemotherapy rhabdomyoblastic differentiation in ERMS; f) Dense pattern ERMS; g) Classic ARMS; h) Solid pattern ARMS, and i) Spindle Cell RMS (SCRMS).

Clinical Data

The study included 28 cases at stage I, 11 cases at stage II, 14 cases at stage III, and 10 cases at stage IV, with the stage unknown in eight cases. The follow-up period ranged from two months to 60 months, with a median follow-up of 13 months. Follow-up details were available for 68 cases (95.8%). Six cases (9.5%) showed recurrences, including three cases of ERMS, two cases of ARMS, and one case of SCRMS. Recurrent tumours exhibited anaplastic morphology with variable cytodifferentiation following chemotherapy. Sixteen cases (25.3%) showed metastasis involving one or two to three metastatic sites, such as lymph nodes (50%), bone (37.5%), lung (28.5%), with one case showing metastasis to the bilateral breasts. Multiple sites of metastasis were observed in four cases, with ARMS being the most common histological subtype to show metastases (50%). Bone marrow involvement was noted in two cases of ARMS.

Three unusual cases were recorded. A 25-year-old girl with retroperitoneal ERMS had a history of Neurofibromatosis (NF) 10 years prior. A 48-year-old woman with uterine ARMS had a history of breast carcinoma six years earlier. A 14-year-old male with thigh ARMS presented with bilateral breast metastasis.

Immunohistochemistry (IHC)

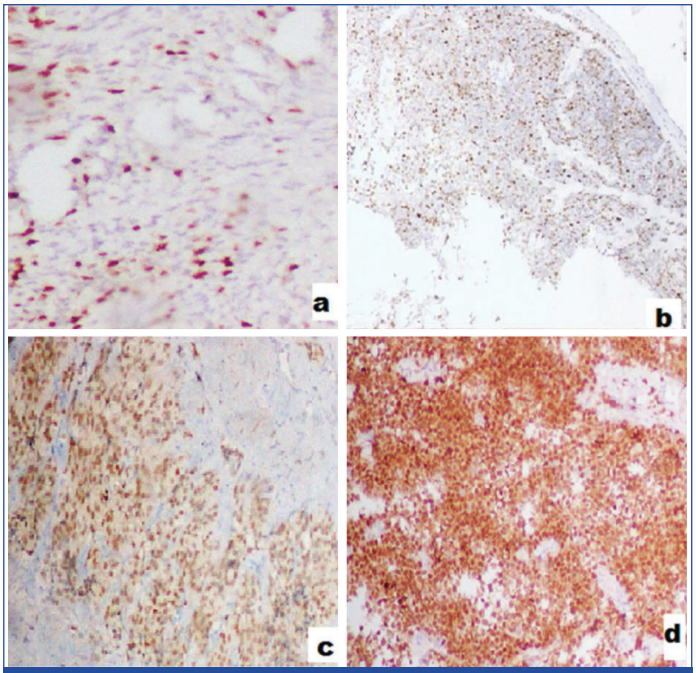
All cases underwent IHC following a morphological diagnosis. A panel of immunohistochemical markers was utilised, and the diagnosis was confirmed by the tumour cells being positive for either myogenin or MyoD1 along with desmin. All cases were positive for desmin (71/71), myogenin (66/66), and MyoD1 (54/54), exhibiting variable immunoreactivity. Aberrant expression of immunomarkers was also observed, which included Pan CK (8/21), synaptophysin (4/15), chromogranin A (3/15), S100 (1/10), SMA (2/10), and CD99 (6/20). Cases were negative for CD34 (0/14) and NKX2.2 (0/21). INI1 was retained in all seven cases evaluated. Myogenin expression was scored and correlated with the histological subtype [Table/Fig-2,3].

Variables	Results	Embryonal RMS (ERMS)	Alveolar RMS	RMS Unclassified	Spindle cell RMS (SCRMS)	Total	p-value
Intensity of myogenin expression	1+ to 3+	26 (100%)	2 (15.4%)	11 (52.4%)	6 (100%)	45 (68.2%)	<0.001
	4+	-	11 (84.6%)	10 (47.6%)	-	21 (31.8%)	
FISH *FOXO1* fusion	Positive	-	13 (100%)	10 (43.4%)	-	23	<0.001
	Negative	10 (100%)	-	8 (34.7%)	1	19	

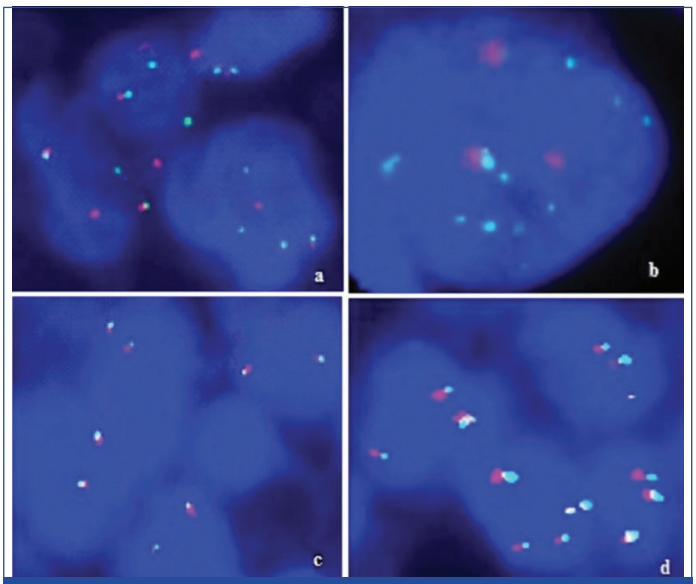
[Table/Fig-2]: Results of myogenin expression and FOXO1 status in histological subtypes.

FOXO1 Fusion Study by FISH

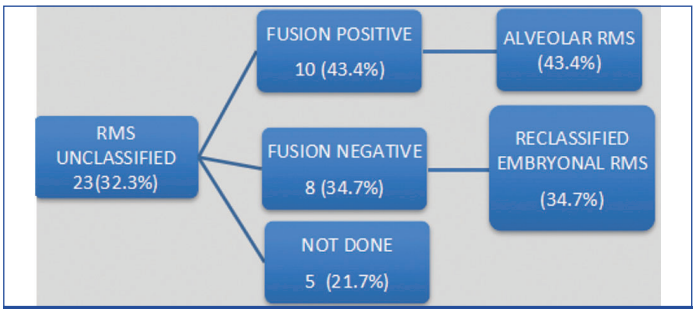
Interphase FISH was conducted in 42 cases. Technical failure was noted in two cases and the procedure was not performed in three cases of the RMS unclassified group due to the patients' unwillingness. FISH was carried out in 10 cases of ERMS, where there was a strong suspicion due to involvement in uncommon sites. Various signal patterns were observed, including typical positive signal patterns indicating rearrangement (1G1O1F) in 11 out of 42 cases, atypical positive signal patterns showing multiple fusions, and break-apart signals (7-8G4-5R3-4F) in 12 out of 42 cases. Typical negative signal patterns, which showed only two fusion signals (2F), were observed in 17 cases. Atypical patterns displaying multiple fusion-only signals (4-5F), indicating polyploidy or amplification, were noted in two cases of ERMS. [Table/Fig-4] shows the *FOXO1* fusion signal patterns. Based on the fusion status, the RMS unclassified group was reclassified, as illustrated in [Table/Fig-5]. [Table/Fig-6] displays the initial distribution of cases based on histomorphology and the final diagnosis after the FISH study.



[Table/Fig-3]: Immunohistochemistry (IHC): Myogenin nuclear staining in tumour cells (x40) a) 1+ (<10% cells) b) 2+ (10-50% cells), c) 3+ (50-90% cells) d) 4+ (>90% cells).



[Table/Fig-4]: Fluorescent insitu hybridisation: FOXO1 break apart-probe: a) 1F1G1O (Positive for rearrangement); b) 7-8G4-5R3-4F- Rearrangement with amplification/ polyploidy (Positive for rearrangement); c) 2F (Negative for rearrangement); d) Multiple fusions- amplification/polyploidy (negative for rearrangement).



[Table/Fig-5]: FOXO1 fusion results and reclassification in RMS unclassified group.

Types	Initial diagnosis	Final diagnosis
Embryonal RMS (ERMS)	28 (42.4%)	28 (42.4%)
Alveolar RMS (ARMS)	13 (18.3%)	23 (32.3%)
Reclassified Embryonal RMS (ERMS)	-	8 (12.1%)
Spindle cell RMS (SCRMS)	7 (9.8%)	7 (9.8%)
Total		66 (96.6%)

[Table/Fig-6]: Reclassification of histological subtype based on *FOXO1* status.

In correlating myogenin expression with FOXO1 fusion, 94.7% of fusion-positive cases had 4+ myogenin expression, while 81.0% of fusion-negative cases exhibited 1+ to 3+ myogenin expression (p-value <0.001). The results are shown in [Table/Fig-7].

Fusion status	Myogenin expression		p-value
	1+ to 3+	4+	
Positive	4 (19.0%)	18 (94.7%)	<0.001
Negative	17 (81.0%)	1 (5.3%)	
Total	21 (100.0%)	19 (100%)	

[Table/Fig-7]: Correlation of myogenin expression and fusion status.

Treatment and Outcome

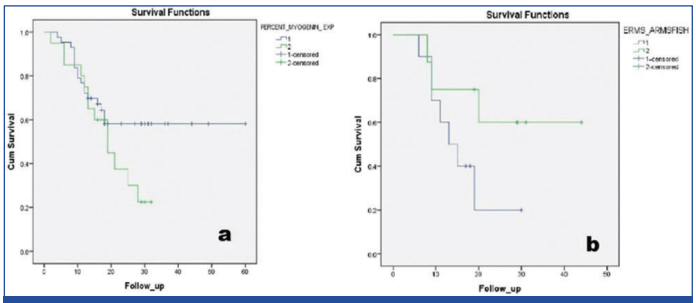
Treatment details were available for 59 out of 71 cases. A multi-modality approach was followed, which included Neoadjuvant Chemotherapy (NACT), surgical excision and Radiotherapy (RT). NACT was administered in 33 out of 59 cases, upfront surgery was performed in 11 out of 59 cases, combined chemotherapy and radiotherapy (CT+RT) were administered in 10 out of 59 cases, and chemotherapy was followed by surgery and adjuvant RT in five out of 59 cases. The chemotherapy protocol followed institutional guidelines: the Inter-group RMS Study (IRS)-4 protocol for adults and the ARST0531 protocol for paediatric patients.

Thirty-five cases completed treatment and remained stable, three were lost to follow-up, and 33 cases died due to loco-regional recurrence, metastasis, disease progression, or defaulting on treatment. Eight cases exhibited loco-regional recurrence, with four cases being ERMS, three ARMS, and one SCRMS. Six out of the eight recurrent cases died despite treatment. The survival analysis with respect to age and gender was conducted in 68 cases, excluding the three lost to follow-up. The OS rate was 51.5%, with a median follow-up of 13 months. Survival rates were higher in children (57.1%, p-value=0.237), males (52.4%, p-value=0.660), tumours involving favourable sites (59.37%, p-value=0.599), and tumours without distant metastasis (54-57% in stages I to III versus 30% in stage IV tumours, p-value=0.494). Tumours with 4+ myogenin expression demonstrated poorer OS compared to tumours with 1+ to 3+ myogenin expression (35% vs. 60.5%), although the data were not statistically significant (p-value=0.143).

Overall, ARMS had the worst OS (26.1%), followed by SCRMS (57.1%), reclassified ERMS (62.5%), and confirmed ERMS (64.2%) (p-value=0.025). In the RMS unclassified subgroup, reclassified ERMS (fusion-negative cases) had better OS than ARMS cases (fusion-positive cases) (62.5% vs. 30.0%) (p-value=0.119). The reclassified ERMS and the confirmed ERMS groups had almost similar survival rates (62.5% vs. 64.2%, respectively). Similarly, in the fusion-positive ARMS group, tumours that showed rearrangement and amplification (n=12) had almost the same OS as tumours showing only rearrangement (n=11) (25% vs. 27.3%, respectively). The survival rates with reference to each parameter are shown in [Table/Fig-8]. [Table/Fig-9] presents the Kaplan-Meier analysis of OS.

S. No.	Variables	Category	No. of cases*	Overall Survival (OS) (%)	p-value
1	Age (years)	0-14	42	24 (57.1%)	0.237
		>14-25	18	8 (44.4%)	
		>25	8	3 (37.5%)	
2	Sex	Male	42	22 (52.4%)	0.660
		Female	26	13 (50.0%)	
3	Site	Favourable	32	19 (59.37%)	0.599
		Unfavourable	34	16 (47.05%)	
4	Stage	1	28	16 (57.1%)	0.494
		2	11	6 (54.5%)	
		3	14	8 (57.1%)	
		4	10	3 (30%)	
5	Myogenin	1+ to 3+	43	26 (60.5%)	0.143
		4+	20	7 (35.0%)	
6	RMS unclassified	Fusion negative	8	5 (62.5%)	0.119
		Fusion positive	10	3 (30.0%)	
7	Final diagnosis	Embryonal RMS (ERMS)	28	18 (64.2%)	**0.025**
		Alveolar RMS (ARMS)	23	6 (26.1%)	
		Reclassified Embryonal RMS (ERMS)	8	5 (62.5%)	
		Spindle Cell RMS (SCRMS)	7	4 (57.1%)	

[Table/Fig-8]: Overall Survival (OS) analysis with various clinical, histopathological, and molecular parameters.
*Cases taken for survival analysis after excluding the lost to follow-up cases and IHC/FISH not done cases



[Table/Fig-9]: Kaplan-Meier survival analysis curve showing Overall Survival (OS): a) Cases with 1+ to 3+myogenin expressions (labelled 1) and 4+ myogenin expression (labelled 2) (p-value=0.143); b) Fusion positive cases (labelled 1) and fusion negative cases (labelled 2) in RMS unclassified group (p-value=0.119).

DISCUSSION

RMS is the most common soft-tissue sarcoma in children, accounting for 3% of all childhood tumours and 50% of all paediatric soft-tissue tumours [11]. In adults, it accounts for approximately 1% of all adult malignancies [12]. The solid variant of ARMS is recognised in the International Classification of Rhabdomyosarcoma (ICR) as a subtype that grows as solid masses of closely aggregated cells, with no or scarcely discernible alveolar arrangement [5]. Conversely, dense ERMS is characterised by mild variation in cell size with a stellate and angulated configuration [5]. In this study, authors classified morphologically overlapping subtypes into confirmed subtypes based on fusion status, investigated myogenin expression and analysed its outcome.

In the current study, a male preponderance was noted, which aligns with previous studies [13,14]. The most commonly affected age group was those under 14 years (60.5%), consistent with findings in other literature [9,13,15]. ERMS was the most common histological subtype (42.4%) across all age groups, followed by ARMS (32.3%), which was in accordance with findings by Amer KM et al., and Davicioni E et al., [12,16]. The head and neck regions, followed by the extremities, were the most commonly affected sites across all age groups, irrespective of histological subtype. This differs from other studies, where extremities were noted as the most common site [15,16]. Regarding histological type and age, the distribution of the primary site varied, with head and neck being more common in ERMS, particularly in children, and extremities being more common in ARMS, often in adults. This aligns with findings from other studies [17-19].

The study revealed an 8.5% relapse rate, with a median time of 17 months from the initial diagnosis. All relapses were loco-regional and occurred across all age groups, which was comparable to findings in other studies [20-23]. The study reported a metastasis rate of 25.3%, which was slightly higher compared to studies by Fu L et al., (21.7%) and Hibbitts E et al., (15.4%) [15,23].

In the present study, a single case of ARMS of the extremity in a 14-year-old male showed metastasis to the bilateral breasts, which

was quite unusual. Upon reviewing the literature, the largest case series found consisted of 19 cases of RMS with breast metastasis [24]. All were female, in contrast to the study case, and all exhibited alveolar histology. The association of the NF1 gene with RMS is well documented in the literature. A retrospective study by Crucis A et al., showed that 16 patients with NF1 had ERMS histology, emphasising that NF1 gene mutation is a risk factor and predisposing factor for ERMS [25].

Regarding IHC, positivity for desmin, myogenin and MyoD1 was observed in all cases of RMS where tested. Rekhi B et al., reported 89.1% positivity for myogenin and 72.2% for MyoD1 [9]. The present study demonstrated 4+ myogenin expression in 84.6% (11/13 cases) of ARMS cases with classic alveolar histology. Similar results were reported by studies conducted by Dias et al., (100%), Kumar et al., (95.8%), Cessna MH et al., (93.7%), and Rekhi B et al., (78.5%) in ARMS [6-9]. This suggests that strong myogenin expression in ARMS can be used as a surrogate test to differentiate this aggressive subtype from other subtypes, particularly in low-income countries where fusion studies are not available. However, the cut-off for high myogenin expression varied across studies, indicating a need for standardised scoring criteria. Additionally, 4+ myogenin expression was associated with poorer overall survival (35% vs 60.5%), which concurred with findings from other studies [7,8,26].

Focal aberrant expression of neuroendocrine markers (23.3%) and focal pan-cytokeratin (38%) expression were noted. Similar findings were reported in studies by Bahrami A et al., [27] and Rekhi B et al., [9]. Other markers with aberrant expression included CD56 (1/9), CD99 (6/20), S100 (1/10), and SMA (2/10). The results were consistent with those of the study by Rekhi B et al., [9]. In the current era, determining a definite morphological subtype and fusion status has become imperative. Moreover, the Children's Oncology Group (COG) has incorporated fusion status into the risk stratification of RMS [23,28].

The relationship between *FOXO1* fusion status and myogenin expression is not fully understood, and studies are limited. In the present study, the correlation between myogenin expression and FOXO1 fusion showed that 94.7% of fusion-positive cases had 4+ myogenin, while 81% of fusion-negative cases displayed 1+ to 3+ expression (p-value <0.001). Present study results were similar to those of studies by Rudzinki ER and Fu L et al., which reaffirm that myogenin expression serves as a surrogate marker for FOXO1 gene fusion status [5,15].

The survival analysis indicated that ARMS (both classic and solid patterns) had the lowest OS (26.1%) among all histological subtypes. The reclassified ERMS group showed almost similar survival rates to the confirmed ERMS group (62.5% vs. 64.2%), thus necessitating the separation of fusion-negative RMS cases from the fusion-positive ARMS category. Rudzinki ER reported an OS of 68% in ARMS and 81% in both original and reclassified ERMS cases [5]. The fusion-negative subgroup demonstrated better survival than the fusion-positive cases (62.5% vs. 30.0%), which was consistent with the study by Rudzinki ER et al., [5].

Atypical signal patterns, such as amplification/polyploidy with rearrangement, were noted in 52.1% of fusion-positive cases, which was concordant with the study by Matsumura T et al., (53.8%) [29]. The survival between cases with rearrangement and amplification and those with only rearrangement was almost similar (25% vs. 27.3%, respectively), in contrast to the findings of Duan F et al., who reported that cases with both rearrangement and amplification had superior survival [30].

Limitation(s)

Certain limitations of the present study include the inability to test fusion-negative cases for other novel gene translocations, such as PAX3-AFX and PAX3-NCOA1, and the challenge of completely excluding ARMS. Additionally, statistically significant data with respect to (OS) could not be obtained due to the shorter follow-up period for the cases.

CONCLUSION(S)

The solid pattern of ARMS and the dense pattern of ERMS do indeed exhibit morphological overlap. Moreover, the limited amount of tumour tissue obtained from needle core biopsies further complicates the subcategorisation of RMS based on morphology alone. Therefore, the present study emphasises the utility of myogenin expression as a valuable surrogate test for distinguishing between these two subtypes. Fusion testing must be conducted in all cases of ARMS and those falling within the unclassified category. This approach is vital for accurate risk stratification and customisation of treatment plans, as the disease's prognosis is significantly influenced by the fusion status.

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