Section: Oncopathology

Original Research Article

THE STUDY OF SPECTRUM OF CYTOGENETIC ABERRATIONS IN EWING SARCOMA WITH CLINICO-PATHOLOGICAL CORRELATION AT A TERTIARY CANCER CENTRE

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Abstract

Background: Ewing sarcoma (ES) are small round cell tumors typically seen in adolescent and childhood. It represents 3% of pediatric tumors. It is an undifferentiated tumor of the bone and soft tissues which may show focal neuroectodermal differentiation. Metastasis is the most important prognostic factor and presence of metastasis substantially reduces the disease-free survival and overall survival of the disease. **Materials and Methods:** Histopathology and IHC slides and blocks of thirty resection specimens were collected from the archives of department of pathology and results of FISH and cytogenetics were corroborated. Study period was 3 years from 01-01-2018 to 31-09-2021. **Result:** Cases of ES more commonly seen in age group <18 years (51.7%) compared to >18 years (48.3%). Commonest site of location was femur (24.1%) followed by humerus (20.7%). Response to chemotherapy showed a significant correlation with metastatic potential of the disease (p=0.02) Cytogenetic aberration shows significant correlation with metastatic potential of the disease (p=0.04) while EWSR1 rearrangement had no statistical significance with metastasis as prognostic factor (p=0.65). **Conclusion:** Our study found a significant correlation between cytogenetic aberrations and metastatic potential of the disease. Since metastasis is a known worrisome prognostic factor in Ewing sarcoma, any parameter which correlates with metastasis, correlates with the prognostication of the disease.

INTRODUCTION

Ewing sarcoma (ES) are small round cell tumors with varying degree of neuro-ectodermal differentiation which was first described by Sir James Ewing in 1921.[1] They constitute the Ewing family of tumors (EFT). It is the second most common bone malignancy in children and adolescents and represents 3 % of pediatric bone tumors.[2] The tumor is less commonly seen in older adults with a smaller peak in incidence in more than 35 years of age. The most common translocation seen is t(11, 22): EWSR1- FLI-1. Other fusion partners seen are t(21,22): EWSR1- ERG1, t(7,22): EWSR1- ETV1 and t(17,22): EWSR1-E1AF can also be rarely seen. Knowledge of variant translocations and additional chromosomal abnormalities helps in diagnosis and prognostication of the disease.[3] ES in about ~50% originates from the diaphysis of long bones and the common locations include pelvis, distal femur, proximal tibia, femoral diaphysis, and proximal humerus.[4,5] 5% of cases can arise from metaphysis. t(11:22) translocation is found in 95% of cases leading to the formation of a fusion protein "EWSR1-FLI1" identified with FISH and PCR which are useful to differentiate Ewing sarcoma from other undifferentiated round cell lesions. All tumors are musculoskeletal tumor society (MSTS) stage IIB or III. 5-year survival is 65-80% for localized disease and 25-40% for metastatic disease while 10-year survival is 60% for localized disease and 30% for metastatic disease.[6] Poor prognostic factors include tumor size greater than 8cm, spine and pelvic location, older age of diagnosis, >18 years, male

gender, elevated lactic dehydrogenase levels >200IUml, anemia and elevated WBC count (indicates extensive disease). p53 mutation in addition to t(11:22) translocation, overexpression of Ki-67 and HER-2/neu are associated with poor prognosis.[7]

Metastases are the most important prognostic factor and its presence is associated with grave prognosis. Lung metastases have a better prognosis than bone/bone marrow metastasis.[8] Skip metastases (same bone involvement) has a better prognosis than metastases to another site. Chemotherapy response with < 90% necrosis is associated with bad prognosis. Symptoms usually present with pain accompanies by fever, often mimicking an infection.[9] Despite presence of established molecular signatures, some of the Ewing sarcoma still exhibits a poor prognosis, hence its imminent to know and understand additional cytogenetic aberrations that may develop in such grave cases. Addressing such aberrations may be important for future diagnosis and prognostication.

MATERIALS AND METHODS

Histopathology and IHC slides and blocks were collected from the archives of department of pathology along with FISH reports for EWSR1 rearrangement. Clinical data collection such as age, sex and clinical presentation were cumulated and follow up was done wherever possible. Clinical details such as pain, swelling, site of lesion, pathological fracture, fever, metastasis at presentation, region of bone involved and serum LDH were procured and frequencies estimated. The histopathology slides, Immunohistochemistry (IHC) slides and FISH reports of all cases were reviewed by three expert oncopathologists. Some of the pathological features were reviewed like mitotic count, presence of atypical mitosis and percentage of necrosis. Frequency of EWSR1 rearrangement in all cases was estimated by FISH. Cytogenetic testing of all cases was done by routine karyotyping. Any new cytogenetic aberrations apart from t(11,22) were addressed, its frequency was estimated and any correlation with known prognostic variables were compared.

RESULTS

The age groups for comparison of parameters were split into <18 years and >/=18 years, as according to literature, age group of ES more than 18 years had a dismal prognosis. The mean age of cases was 15 years. Sixteen out of thirty cases were in the age group less than 18 years and fourteen cases were more than 18 years. Of the thirty cases of ES, a majority were males (72.4%) compared to females (27.6%).

1. Tumor Location

Preponderance of cases of ES had its epicenter of lesion from femur (24.1%) and humerus (20.7%) while rare cases of ES were seen arising from calcaneum and pelvis.

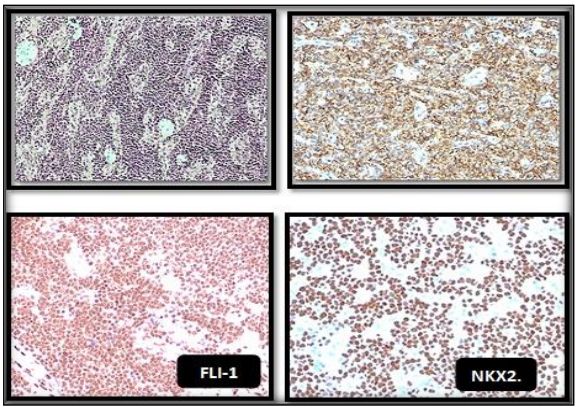

Figure 1: Photomicrographs exhibiting the histopathological and immunohistochemical (Positive for CD99/FLI-1/NKX2.2) staining of cases of ES

Large number of cases arose from the diaphysis of long bones (36.7%) followed by meta- diaphysis (26.7%). Bulk of cases displayed tumor dimensions more than 8cm (55.2%). Majority of cases of ES had a predominant cortical (72.4%) epicenter of lesion followed by medullary (17.2%). Swelling (93.1%) was the common presentation in predominant cases followed by pain (51.7%). Most cases of ES histopathologically displayed tumor necrosis less than 90% (75.9%). Most cases did not present with metastasis (73.3%). Lung was the most common metastatic site involved (16.7%). EWSR1 rearrangement was seen in 70.2% cases,

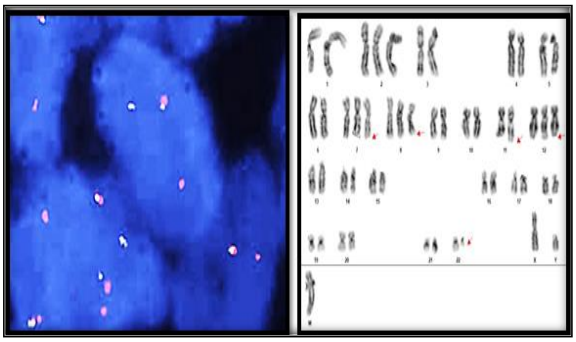

Figure 2: Photographs illustrating 1. Break apart FISH for the characteristic EWSR1 rearrangement: nuc ish(EWSR1x2)(5'EWSR1 sep 3'EWSR1x1)[200] and 2.The cytogenetic anomalies of a case of ES:51 XY,+2,+7,+8,t(11;22)(q23;q11.2),+12,+mar

2. Correlation of Response to chemotherapy with metastasis of the disease

There was a statistical correlation compared between response to chemotherapy and metastasis at presentation. Cases which presented with metastasis generally had a poor response to chemotherapy than with cases which did not present with metastasis.

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Hence response to chemotherapy was considered a robust prognostic and predictive marker for ES.

Table 1: Correlation between response to chemotherapy and metastatic potential of the disease

	Response to chemotherapy	<90% response	>90% response
Metastasis			
Present		8 (100%)	0 (0%)
Absent		16 (72.7%)	6 (27.2%)
			P: 0.02

3: Correlation of EWSR1 rearrangement with metastasis at presentation

There was no statistical significance comparing EWSR1 rearrangement and metastasis of the disease at presentation, hence EWSR1 rearrangement may not be considered as a prognostic variable for ES, nevertheless it's a principal molecular change in diagnosis.

Table 2: Correlation between EWSR1reaarangement and metastatic potential of the disease

Diseases	Mild	Moderate	Severe
Bipolar	30(60%)	9(18%)	11(22%)
Dementia	6(12%)	20(40%)	24(48%)
Schizophernia	5(10%)	19(38%)	26%(52%)
Substance abuse	7(14%)	15(30%)	28(56%)

4: Correlation of additional Cytogenetic aberrations with metastasis at presentation.

Presence of additional cytogenetic aberrations demonstrated by the tumor strongly correlated with metastatic presentation of the disease. No statistical significance was obtained to clearly demarcate the type of cytogenetic abnormality considering the truncated number of cases. However it was seen that trisomy 8 (33.3%) formed the bulk of cytogenetic abnormal ES cases followed by trisomy 20 (22.2%).

Table 3: Correlation between additional cytogenetic aberrations and metastasis

	Additional Cytogenetic aberrations	Present	Absent
Metastasis			
Present		7 (87.5%)	1 (12.5%)
Absent		2 (10%)	20 (90.9%)
			P: 0.04

4: 5: The cytogenetic aberrations noted in cases of ES

Various cytogenetic aberrations noted in the present study summarized in the table below.

Table 4: Cytogenetic Aberrations in ES cases

Ewing sarcoma cases with cytogenetic aberrations	Additional cytogenetic aberrations
Case 1	+8
Case 2	+8, +13, +2
Case 3	t(1,16), t(5,9) +17, +18, +21
Case 4	-22, +20, +8
Case 5	+2, +5
Case 6	+7, +2
Case 7	-16
Case 8	+20
Case 9	+12

DISCUSSION

Kullendorf et al recorded clonal chromosome aberrations in eighteen patients, seventeen of whom had the characteristic t(11;22)(q24;q12) or variants thereof. The most frequent secondary change was +8, followed by +12, +2, +5, +9, +15, and gain of material from the long and short arms of chromosome 1. The only recurrent secondary change that was restricted to tumors from the ten patients that were dead at latest follow-up was gain of 1q material. Furthermore, all three patients with tumors with chromosome numbers over 50 had died, and the only patient with a tumor karyotype lacking chromosome 22 rearrangements was alive without evidence of disease.[14] Our study also showed +8 as the most frequent cytogenetic abnormality which was accorded to several studies

M Zielenska et al studied gains of chromosomes 8 and 12 detected, by interphase FISH, in 48% (10 of 21) and 38% (6 of 16) of the tumors, respectively was not significant with respect to treatment response. Statistical analysis revealed that the presence of additional secondary structural chromosomal aberrations was associated with an unfavorable outcome (P = 0.0034 as an independent prognostic value as an unfavorable marker). Presence of metastasis at diagnosis also was found to be associated with poor outcome (P = 0.0131). Spectral karyotyping analysis was shown to facilitate the detection of more complex structural chromosomal aberrations in a representative ES

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tumor.[15] In our study only 9 cases exhibited cytogenetic abnormalities and hence we were not able to confidently attribute a single cytogenetic anomaly with prognosis of the disease, thereby a collective cytogenetic consideration was made. Paul Roberts et al emphasized that trisomy 20 was associated with a worse overall survival and disease-free survival and that there was no differences in outcome associated with other recurrent trisomies of 2, 5, 7, 8, or 12 or the common recurrent secondary structural rearrangements (deletions of 1p36, 9p12, 17p13, and 16q, and gain of 1q), although numbers were small.[11] CM Hattinger et al stated that gain of chromosome 8 occurred in 52% of Ewing tumors and was associated with poor prognosis. Gain of 1q was associated with adverse overall survival and event-free survival in all patients, irrespective of whether the tumor was localized or disseminated. Loss of 16q and t(1,16) was a significant predictive factor for adverse overall survival in all patients (P=0.008) and was associated with disseminated disease at diagnosis. Gain of chromosome 12 was associated with adverse event-free survival (P=0.009) in patients with localized disease.[12] Our study had the same conclusions that cytogenetic aberrations have a strong correlation with metastatic potential of the disease and Trisomy 20 and Trisomy 8 was the most common cytogenetic aberrations noted.

G Bacci et al stated that there is no significant correlation with EWSR1 rearrangement and disease-free survival / overall survival. 65% of cases of Ewing sarcoma showed EWSR1 rearrangement and strong prognostic correlation was noted with other parameters such as poor response to chemotherapy induced necrosis, presence of fever, anemia, age more than 18 years and elevated LDH levels.[13] Our study concluded that there is no significant correlation with EWSR1 rearrangement and metastatic potential of the disease.

CONCLUSION

Our study found a significant correlation between cytogenetic aberrations and metastatic potential of the disease. Since metastasis is a known worrisome prognostic factor in Ewing sarcoma, any parameter which correlates with metastasis, correlates with the prognostication of the disease. For Disease Free Survival and Overall Survival, 5-10 year follow up was necessary in pediatric population and 3 years follow up in adults or >/= 50% cases should show significant events. Since such events were not obtained in our study, our study had to rely on known prognostic variables, hence metastasis was the best variable considered for prognostication of the disease.

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