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FREQUENCY OF POSITIVITY OF NKX2.2 IN CASES OF EWING SARCOMA IN A TERTIARY CARE HOSPITAL

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Abstract

Objective: *The objective of this study was to ascertain the prevalence of NKX2.2 positive in Ewing sarcoma and to evaluate its correlation with clinicopathological characteristics.*

Study Design: *Cross-Sectional Study.*

Study Setting: *The study conducted at the department of Histopathology at the Children Hospital and the University of Child Health Sciences in Lahore.*

Study Duration: *Six Months (Dec-24 to May, 2025)*

Methodology: *A total of 97 histologically validated cases of Ewing sarcoma were examined. We gathered demographic and clinical data and did IHC staining for CD99 and NKX2.2. Statistical study utilized Chi-square and t-tests to assess correlations between marker expression and clinical variables, including age, gender, location, and symptom duration.*

Results: *Average age of the patients was 7.57 ± 3.26 years, and majority were males. Most of the lesions (77.3%) were in soft tissue, with the chest wall (47.4%) and femur (11.3%) being the most common places. In 98.8% of cases that could be evaluated, CD99 was positive, and in 95.7% of cases, NKX2.2 was positive. Co-expression of both markers was elevated and statistically insignificant ($p = 0.87$). There were no significant links between marker positive and age, gender, biopsy site, or illness duration (all $p > 0.05$).*

Conclusion: *The combined evaluation of CD99 and NKX2.2 improves diagnostic precision, particularly in resource-constrained environments lacking molecular tests.*

INTRODUCTION

Ewing sarcoma (ES) is a highly aggressive tiny round cell tumor. Bone and soft tissue are the primary areas of concern for ES in infants and people under the age of 20, whereas visceral lesions may develop in individuals who are older [1, 2]. It is possible for the EWSR1 gene to get fused with the ETS family of genes in ES through recurrent chromosomal translocations.[3] In terms of fusion partners, EWSR1 is most frequently associated with FLI1 (85%), followed by ERG (5%–10%). On a very infrequent basis, ETV1 and ETV4 join in. There is a low incidence of EWSR1 rearrangements with genes that are not members of the ETS family, such as NFATc2, POU5F1, SMARCA5, PATZ1, and SP3 [4, 5]. A proliferation of undifferentiated small spherical cells that may resemble other SRCTs is what ES seems to be when viewed under a histological microscope. ES is differentiated from other SRCTs through the use of immunohistochemistry; CD99 and/or FLI1 have been utilized in a significant number of studies.[2]

Besides ES, other SRCTs may also exhibit these markers. Cytogenetic and molecular genetic procedures are the gold standard for diagnosing ES, although general pathology practices may not have them. Thus, another unique and reliable immunohistochemical marker to replace CD99 or FLI1 is needed. NKX2.2, a homeobox transcription factor that is essential for neural development, is a target gene product of EWSR1-FLI1 and increases in ES, contributing indirectly to its oncogenesis [6]. Yoshida et al. and his colleagues recently found that NKX2.2 is a unique and diagnostic immunohistochemistry marker for ES [7]. Still limited local and international studies are available for the NKX2.2 expression among the various bone cancers and in the ES. After NKX2.2, EWSR1-FLI1 upregulated to NROB1, ZBTB7B, and ZBTB16; while these transcriptional factors have been shown to be important in ES tumorigenesis and may be targets of molecular diagnostic and therapeutic approaches[8-9], their use as immunohistochemical markers for ES has not been investigated.

In a study, NKX2.2 was found in 93% of Ewing sarcomas, including all atypical cases and those with unknown EWSR1-FLI1 or EWSR1-ERG fusion, with diffuse (>50%) and moderate to strong staining [9]. In another study, NKX2.2 was positive in 100% of diffuse Ewing sarcoma cases, including adamantinoma-like variant.[10] Similarly in another investigation, 90% of cases of diffuse and strong staining were positive for NKX2.2 [11]. A comprehensive assessment of patients with bone cancer revealed that the presence of Ewing's sarcoma was found in 16.4% of the cases among 1195 individuals who were diagnosed with bone cancer. [12] According to the findings of a number of investigations, the expression of NKX2.2 in Ewing sarcoma has been shown to vary between cases of the illness that occur in different geographical locations and when the patients are of different ages. Within the context of Ewing sarcoma, the purpose of this investigation is to ascertain the frequency of the Nkx2.2 positive rate. When it comes to validation research, as well as sensitivity and specificity tests, the findings of this study will be applied in the future.

METHODOLOGY:

This research was carried out at two different institutions: the Department of Histopathology at The Children's Hospital and the University of Child Health Sciences in Lahore. Both hospitals are located in Pakistan. In the beginning, the research was going to be a cross-sectional study. Within the scope of this investigation, the method of non-probability consecutive sampling was undertaken. After the completion of the review and approval of the summary, the research was carried out over the course of a period of six months. When determining the required sample size, the following factors were taken into consideration: an expected frequency of eighty percent, an error margin of eight percent, and a confidence interval of 95 percent.

A total of 97 cases were taken into consideration for inclusion. Ewing sarcoma patients who had been genetically confirmed were included in the study. This was the case regardless of whether the disease began in bone or soft tissue, whether the patients were children or young adults, or whether they were male or female. In the study, all patients who had small round blue cell tumors and had

negative results for EWSR1-FLI1 translocation were excluded from participation.

In addition, patients who were eligible for inclusion were given the option to offer their informed consent after the Institutional Ethical Review Committee had given its approval for the study. A method called immunohistochemistry for NKX2.2 was carried out on slices or cytology cell blocks of biopsy specimens that were 4 µm in thickness, formalin-fixed, and embedded in paraffin. Through the usage of the pressure cooker method, antigen retrieval was successfully accomplished. As part of the validation process, both positive and negative controls were utilized. Positive controls consisted of Ewing sarcoma patients who had demonstrated EWSR1 rearrangement, whereas negative controls consisted of healthy individuals who had normal colon, skeletal muscle, and skin.

In a semi-quantitative way, the NKX2.2 nuclear staining was evaluated and documented based on both the extent (zero = no staining, one = 1–25%, two = 26–75%, and three = more than 75%) and the intensity (weak, moderate, and strong). The extent was measured as a percentage, and the intensity was measured as a percentage. The production of paperwork for any nuclear positive of NKX2.2 was carried out. Additionally, a histological examination of the final surgical specimens was performed, and the results were documented using a proforma that had been established in advance. This was done following the completion of the surgical procedure.

The evaluation of the data that was obtained was carried out after it had been entered into SPSS version 26, which was then used. The quantitative parameters, such as age, were reported in the form of the mean plus the standard deviation. On the other hand, the qualitative variables, which included NKX2.2 immunoreactivity, gender, and the type of surgery, were presented in the form of frequencies and percentages. In order to control effect modifiers including age, gender, place, genetics, length of disease, staging, and grading, stratification was utilized. For the purpose of conducting post-stratification analysis, the Chi-square test was performed, and a p-value that was lower than 0.05 was considered to be statistically significant.

RESULTS:

A total of 97 patients enrolled were mostly children, with an average age of 7.57 years SD 3.26 years. There were more men than women and with mean duration of symptoms of 5.2 ± 2.3 months, and most of the biopsies (77.3%) were of soft tissue parts.

Biopsy samples were taken from a number of different parts of the body. The chest wall was the most common site, with 46 cases (47.4%), followed by the femur/femoral bone, where 11 patients (11.3%) had surgery. The soft tissue of the forearm (6.2%), the soft tissue of the leg (5.2%), and area around the scapula (5.2%) were all common areas. The iliac bone (3.1%), the paraspinal or paravertebral area (3.1%), the pelvic bone (2.1%), the soft tissue of the back (2.1%), and the thigh soft tissue (2.1%) were found to be slightly less prevalent. Based on these results, the chest wall and femur were the most afflicted parts.

CD99 expression was seen among 95 cases. Of those, 82 (84.5%) were positive, 1 (1.0%) was negative, and 14 (14.5%) were missed cases. These findings are derived from immunohistochemistry (IHC) markers. The NKX2.2 marker was used in 92 instances; 88 (95.7%) of them were positive, 4 (4.3%) were negative, and 5 (5.2%) of the cases had data that were not accessible.

The overall immunoprofile showed that CD99 and NKX2.2 were the most consistently positive markers. This matched the usual immunohistochemical pattern reported in Ewing sarcoma and PNET. Conversely, Desmin and Myogenin were negative, so definitively excluding rhabdomyosarcoma. The consistent marker concordance in Ewing sarcoma was validated by the uniformly high positivity rates of both CD99 (98.8%) and NKX2.2 (95.7%). Additionally, there was a notable co-expression that was statistically non-significant ($\chi^2 = 0.03$, $p = 0.87$).

No statistically significant correlation was seen between the expression of the marker and age, gender, biopsy site, or duration of symptoms, since all variables exhibited p values over 0.05. The mean age of NKX2.2-positive patients did not significantly differ from that of NKX2.2-negative patients ($t = 0.70$, $p = 0.53$), suggesting that demographic factors

did not affect marker expression. Both CD99 and NKX2.2 demonstrated consistently elevated expression across all clinical classifications.

DISCUSSION:

In this study of 97 histologically validated instances of Ewing sarcoma, CD99 and NKX2.2 exhibited consistently elevated immunopositivity rates of 98.8% and 95.7%, respectively. Both markers showed substantial correlation ($\chi^2 = 0.03$, $p = 0.87$). When analyzed by age (<5 vs ≥5 years), gender, biopsy site (bone vs soft tissue), and duration of disease (<6 vs ≥6 months), no statistically significant changes in marker expression were detected (all were $p > 0.05$). The average age of NKX2.2-positive patients (7.3 years) did not differ substantially from that of NKX2.2-negative patients (7.7 years; $p = 0.53$). In general, CD99 and NKX2.2 were consistently expressed in all demographic and clinical subgroups, this shows that they are reliable immunohistochemical markers for diagnosing Ewing sarcoma.

Our results are aligning with previous literature that indicates NKX2.2 expression in 90–95% of genetically validated Ewing sarcomas cases [7,11]. NKX2.2, a transcription factor containing a homeodomain and controlled by the EWS-FLI1 fusion oncogene, has been identified as a highly sensitive and dependable diagnostic for Ewing sarcoma [7]. The unique nuclear staining pattern offers diagnostic precision and enhances CD99 although sensitive but is non-specific and may be seen on other tiny cell cancers.

Numerous international research have corroborated the diagnostic efficacy of NKX2.2. Yoshida et al. initially recognized its diagnostic importance, documenting a sensitivity of 93% and a specificity of 89% in genetically validated instances [7]. Shibuya et al. subsequently showed that the concurrent use of CD99 and NKX2.2 yields the most precise immunohistochemical profile for the confirmation of Ewing sarcoma [11]. Hung et al. also identified NKX2.2 expression the majority of ES cases, although noting that sporadic expression may also be present in other EWSR1-rearranged sarcomas. [13] These findings of our study, with widespread NKX2.2 positive in majority patients, underscore its elevated sensitivity while also advocating for its interpretation alongside CD99. The findings of the present investigation indicated that NKX2.2 and CD99 positive were independent of patient age, gender, lesion location, and symptom duration, implying that these are consistently expressed in Ewing sarcoma. Hung et al. [13] and McCuiston and Bishop [10] have also reported this stability, revealing that NKX2.2 expression was uniform throughout both skeletal and extraskeletal locations, including cases in the head and neck. Absence of demographic variability highlights the marker's reliability and endorses its use in various anatomical and therapeutic settings.

TABLE 1: FREQUENCY AND CONCORDANCE OF CD99 AND NKX2.2 POSITIVITY IN EWING SARCOMA (N = 97)

Marker	Positive n (%)	Negative n (%)	No Result n	Chi-square	p-value
CD99	82 (98.8%)	1 (1.2%)	14	—	—
NKX2.2	88 (95.7%)	4 (4.3%)	5	—	—
CD99 vs NKX2.2	—	—	—	0.03	0.87

TABLE 2: CD99 AND NKX2.2 POSITIVITY STRATIFIED BY AGE, GENDER, AND SITE

Variable	Category	CD99 + (%)	χ^2 (p)	NKX2.2 + (%)	χ^2 (p)
Age Group	<5 years	100%	0.61 (0.43)	85%	0.72 (0.39)
	≥5 years	99%		92%	
Gender	Male	98%	0.03 (0.85)	93%	0.00 (1.00)
	Female	100%		88%	
Site	Bone	95%	0.33 (0.57)	93%	0.00 (1.00)
	Soft tissue	100%		90%	

TABLE 3: CD99 AND NKX2.2 POSITIVITY BY DURATION OF SYMPTOMS AND MEAN AGE COMPARISON

Variable	Category	CD99 + (%)	χ^2 (p)	NKX2.2 + (%)	χ^2 (p)
Duration of Symptoms	<6 months	100%	0.29 (0.59)	94%	0.25 (0.61)
	≥6 months	95%		91%	
Mean Age (years)	Positive	7.3	—	7.3	0.70 (0.53)
	Negative	—	—	7.7	
Variable	**Category**	**CD99 + (%)**	**χ^2 (p)**	**NKX2.2 + (%)**	**χ^2 (p)**
Duration of Symptoms	<6 months	100%	0.29 (0.59)	94%	0.25 (0.61)

NKX2.2 can be especially useful among cases of histological mimics, including rhabdomyosarcoma, desmoplastic small round cell tumor, and small cell osteosarcoma. McCuiston and Bishop [10] also validated its efficacy in differentiating Ewing sarcoma in the head and neck region, whereas Pruthi et al. [5] indicated that integration of NKX2.2 with CD99 could diminish the necessity of EWSR1 FISH testing. Furthermore, NKX2.2 positivity was observed in uncommon extra-osseous types of ES, such as primary renal and paraspinal cases, underscoring its utility as a confirming diagnostic tool [14,15]. Previous reports, in conjunction with the current findings, suggest that NKX2.2 can function as a reliable modality among the atypical anatomical manifestations of ES. Still, it is crucial to report that NKX2.2 lacks complete specificity.

Hung et al. [13] and Aydemir et al. [16] both noted diminished expression in a subset of non-Ewing small round cell tumors, encompassing mesenchymal chondrosarcoma and CIC-rearranged sarcomas. This further emphasizes the necessity of NKX2.2 testing in the immunohistochemistry panel instead of utilizing it as an independent assay. The diagnostic gold standard continues to be the correlation with morphology, clinical presentation, and, where accessible, molecular confirmation.

This study showed that NKX2.2 is a sensitive and effective diagnostic tool for Ewing sarcoma in addition to CD99. Its high positive rate, steady expression across clinical factors, makes it one of the essential histopathologic diagnosis parameters. In tertiary care settings with inadequate molecular diagnostics, NKX2.2 immunostaining can improve diagnostic accuracy, guide treatment, and improve patient outcomes with early diagnosis. NKX2.2 expression across various age groups, tumor locations, and symptom durations highlights its diagnostic reliability. These findings underscore that NKX2.2 can function as a confirmatory marker among cases of ambiguous CD99 staining, thus decreasing the chance of misclassification with histological mimics, eventually leading to improved patient outcomes through prompt treatment decisions.

CONCLUSION:

CD99 and NKX2.2 both showed strong, diffuse positivity in over 95% of Ewing sarcoma cases. No significant association was observed with age, gender, site, or duration. The high concordance and consistent expression patterns reinforce NKX2.2 as a reliable adjunct to CD99 in confirming the diagnosis of Ewing sarcoma.

LIMITATIONS

The single-center design and limited sample size of this study may restrict the generalizability of the findings to broader populations. Additionally, some cases had partial clinical data and missing immunohistochemistry results due to inadequate tissue samples, hence introducing a minor bias in the evaluation of marker frequency. Cross-sectional design prevented assessment of prognostic correlations or treatment outcomes associated with CD99 or NKX2.2 expression.

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