IMMUNOHISTOCHEMICAL EXPRESSION OF TRANSDUCER LIKE ENHANCER OF SPLIT 1 (TLE-1) IN CASES OF SYNOVIAL SARCOMA

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ABSTRACT

Objective: To determine the immunohistochemical (IHC) expression of transducer like enhancer of split 1 (TLE-1) in diagnosed cases of synovial sarcoma (SS).

Study Design: Cross sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from Jan 2017 to Jun 2018.

Methodology: In our study, 60 cases of SS diagnosed on hematoxylin & eosin (H&E) morphology, IHC expression of CD99 and epithelial membrane antigen (EMA) were retrieved from archive of Histopathology department AFIP Rawalpindi. Patient's age, gender, tumor site and histologic type were noted. IHC for TLE-1 was applied and results were recorded. Data was analyzed using SPSS version 22.

Results: A total of 60 cases were included in which 33 were males and 27 were females. The male to female ratio was 1.2:1. Mean age of the study patients was 36.6 ± 15 years. SS was found be more common in soft tissues of limbs/ extremities around tendons sheaths (76%); other sites being lungs, head & neck and retroperitoneum. Out of 60 cases of SS, 42 (70%) cases were monophasic, while 15 (25%) and 3 (5%) cases were biphasic and poorly differentiated SS, respectively. A total of 90% samples (n=54) showed immunoreactivity for TLE-1 (p=0.023) out of which 61% samples (n=33) had diffuse (2+) nuclear staining while 21 (39%) showed weak to moderate nuclear staining for TLE-1. However, 6 (10%) cases did not show nuclear staining for TLE-1.

Conclusion: TLE-1 was found to be a novel marker which was extremely helpful in diagnosis of SS, especially in cases where diagnostic challenge arises when other spindle cell neoplasms are considered in the differential diagnosis and in laboratory set ups where advanced facilities of molecular testing by fluorescent in situ hybridization (FISH) is not available.

Keywords: Immunohistochemistry, Spindle cell sarcomas, Synovial sarcoma, Transducer like enhancer of split 1 (TLE-1).

INTRODUCTION

Synovial sarcoma (SS) is a clinically and morphologically well-defined entity which commonly occurs in young to middle age adults[1]. It represents 5-10% of all soft tissue sarcomas[2]. A study reveals that 15% cases occur in children younger than age 15, while 40% cases occur after age of 55[3]. The tumors are usually of mesodermal origin, although a few are derived from neuroectoderm, and they are biologically distinct from the more common epithelial malignancies[3]. SS is extremely uncommon in joint cavities and is encountered in areas with no apparent relation to synovial structures like mediastinum and retro peritoneum[4]. SS is a grade-III neoplasm by definition and shows aggressive biologic behavior. Luckily it is responsive to treatment modalities like chemo-therapy. So, an accurate and early diagnosis is vital for better management and prognosis.

Histologically, SS encompasses three subtypes: biphasic, monophasic and a poorly differentiated. Biphasic SS has distinct epithelial and spindle cell components in varying proportions. Poorly differentiated SS has small, round blue cells, hence, falling in the differential diagnosis of round blue cell tumor. Diagnosing biphasic SS is

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generally straightforward, owing to distinctive histologic features.

Differentiating monophasic SS from other spindle cells neoplasms is a diagnostic challenge. Several IHC panels are available to distinguish it from histological mimickers but due to scant biopsy tissue, fixation artifacts and focal expression of epithelial markers, the diagnosis becomes even more challenging. Previously, no single sensitive or specific marker was available for SS and diagnosis were made upon exclusion of other mimickers[4]. However studies suggest TLE-1 is a highly sensitive marker for SS and it is not expressed in non-SS neoplasms[5].

SS is characterized by the translocation t(X;18) that produces the fusion oncogenes SYT-SSX. This translocation involves the fusion of the SYT gene on chromosome[18], and either the SSX1 or SSX2 gene on the X chromosome, or rarely with SSX4.5 Hence, molecular detection of SYT-SSX fusion gene using fluorescent in situ hybridization (FISH) is considered to be the diagnostic gold standard[5,6].

The molecular techniques are costly, not widely available in laboratories, and often require fresh or frozen tissue. Thus, there is a need felt for recognition of less expensive and widely available immunohistochemistry marker for diagnosis of SS[7].

Transducer like enhancer of split 1 (TLE-1) is one of 4 TLE genes that encode human transcriptional repressors homologous to the Drosophila corepressor groucho. TLE proteins are expressed in embryogenesis where they are involved in developmental processes including neurogenesis, body patterning, and hematopoesis particularly TLE1. For that reason, immunostains for TLE1 protein may be helpful in the recognition of SS[8].

Of late, a number of studies have been done evaluating the expression of TLE-1 in cases of SS, hence critically analyzing its utility in efficacy as a sole diagnostic marker[7-12].

The objective of this study was to evaluate frequency of expression of TLE-1 in cases of SS using immuno-histochemistry. This study which will help in differentiation of SS from other spindle cells neoplasms considered in its differential diagnosis.

METHODOLOGY

This cross-sectional study was carried out in the department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from January 2017 to June 2018 after taking approval of Institutional Review Board and Institutional Ethical Committee, AFIP Rawalpindi.

A total of sixty cases of SS diagnosed on morphological examination and IHC expression of epithelial membrane antigen (EMA) and CD 99 were selected by non-probability consecutive sampling. All specimens of synovial sarcoma irrespective of age of patient, histological type and grade of the tumor, were included. Poorly fixed specimens, specimens with scanty tissue and aberrant IHC results due to fixation artifacts were excluded from the study.

Formalin fixed and paraffin embedded tissue blocks were sectioned at 3µm thickness and deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. Heat induced epitope retrieval in Tris/EDTA buffer at pH 9.0 buffer was done and applied on ready to use primary antibody TLE1 of BioSB kit (catalogue number BSB 2315) on LEICA Bond III as per the manufacturer's guidelines. Nuclear immunoreactivity was taken as positive, based of proportion of staining, no staining was 0 negative, 1+ mild to moderate (5-50% of tumor cells) and 2+ strong positive (>50% of tumor cells)[3]. Quality control was ensured and positive control was applied with each section.

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 22.0 (BM Corp., Armonk, NY, USA). Mean and SD was calculated for quantitative variables. Frequencies, and percentages were calculated for qualitative variables for Associations. Chi square test was applied to calculate associations between

age and SS, site and SS, gender and SS, positivity of TLE-1 and type of SS. *p*-value ≤0.05 was considered significant.

RESULTS

Mean age was 36.6 ± 15 years. Cases were between 13 and 72 years of age and range was 59 years. It was found to be more prevalent between

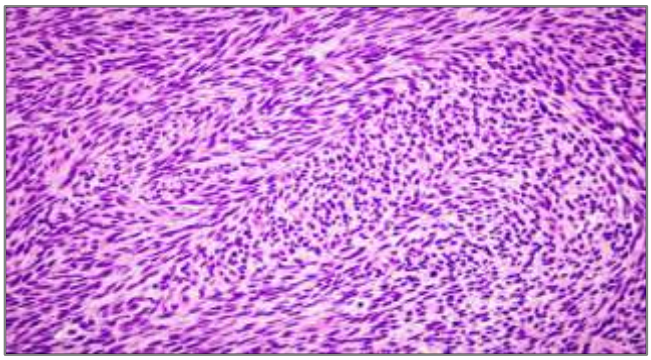

Figure-1: H&E Morphology of Monophasic Synovial Sarcoma in a male patient aged 27 Years.

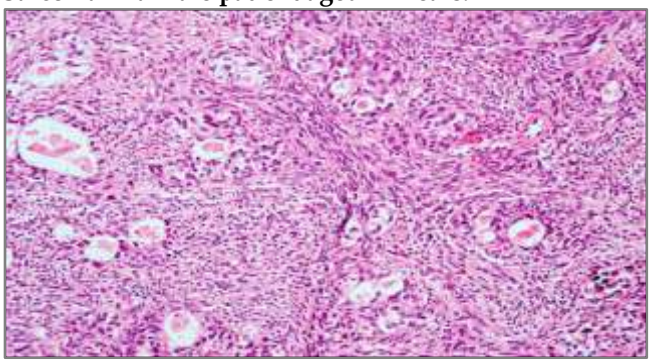

Figure-2: H & E Morphology of a Biphasic Synovial Sarcoma in a female patient of 47 years.

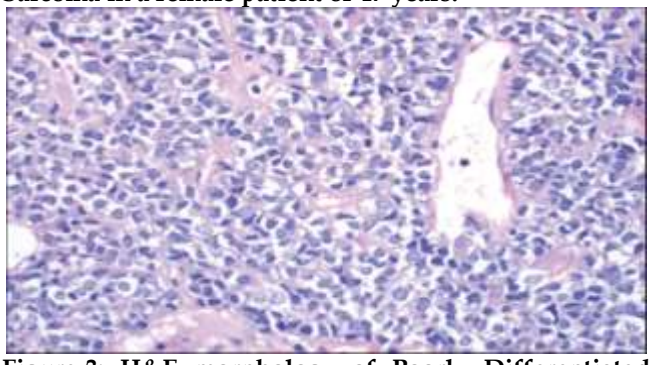

Figure-3: H&E morphology of Poorly Differentiated Synovial Sarcoma in a young male patient of 23 years.

20-40 years of age 32 (53%). Out of 60 cases, 33 (55%) were males and 27 (45%) were females. Male to female ratio was 1.2:1. Majority of the cases 46 (76%) were seen in bone and soft tissues of extremities followed by lung 5 (8%) and retroperitoneum 2 (3%) as other major sites. A total of 42 (70%) cases were of monophasic SS (fig-1), while 15 (25%) were of biphasic (fig-2) and 3 (5%) cases of poorly differentiated SS (fig-3), respectively (fig-4). Out of 60 samples, 90% samples (n=54) showed positive immuno-reactivity for TLE-1 (fig-5) (*p*=0.023). Only 6 (10%) cases did not show nuclear staining for TLE-1. A total of 40 (95%) cases of monophasic SS were positive for TLE-1 and 11 (73%) cases of biphasic SS are positive for TLE-1. All 3 cases of poorly differen-

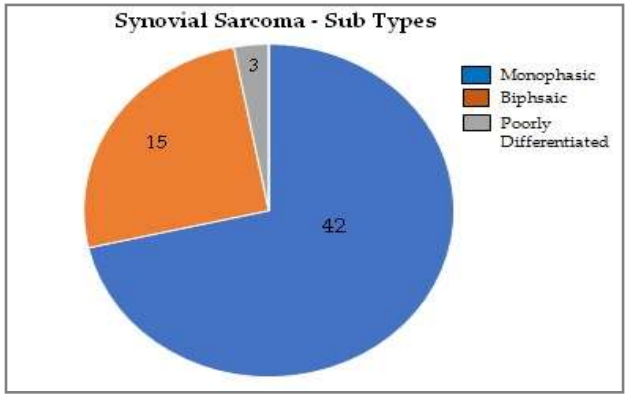

Figure-4: Synovial sarcoma subtypes (n=60).

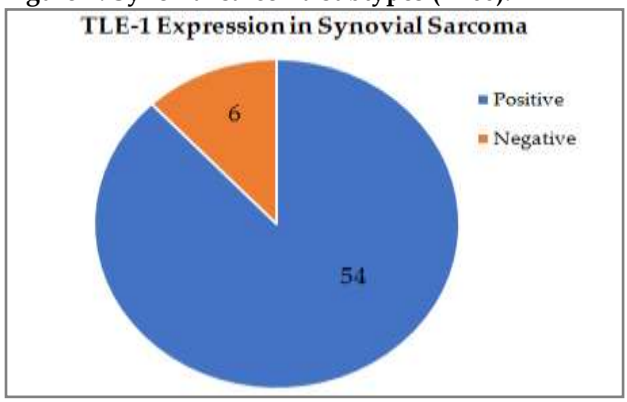

Figure-5: Expression of TLE-1 in synovial sarcoma (n=60).

tiated synovialsarcomas showed immunore activity to TLE-1 (table). Strong nuclear positivity (2+) for TLE-1 was seen in 10 (90%) biphasic and 22 (55%) monophasic SS cases. Mild to moderate nuclear staining (1+) for TLE-1 was seen in 1 (9%) cases of biphasic and 18 (45%) cases of monophasic SS. Two cases of poorly differentiated subset were mild to moderate positive (1+) for TLE-1 and 1 case was diffusely positive for TLE-1 (2+). Two cases of monophasic SS and 4 cases of

biphasic SS were negative for immunoreactivity to TLE-1 (*p*-value=0.023) (table).

DISCUSSION

In our study the mean age of the 60 cases was 36.6 ± 15 years. The age of patients ranged between 13 and 72 years. Our findings were in concordance with a study conducted on 121 cases of SS in which the range was 9 to 74 years[1]. In study conducted in Mexico 15 cases of pleuro-pulmonary SS were taken and the mean age was

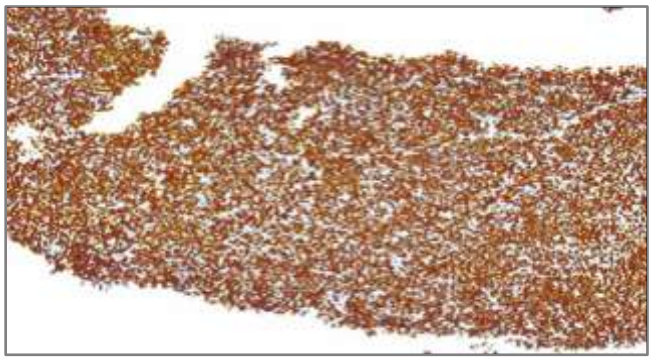

Figure-6: Diffuse Staining (2+) TLE-1 in a mono-phasic synovial sarcoma.

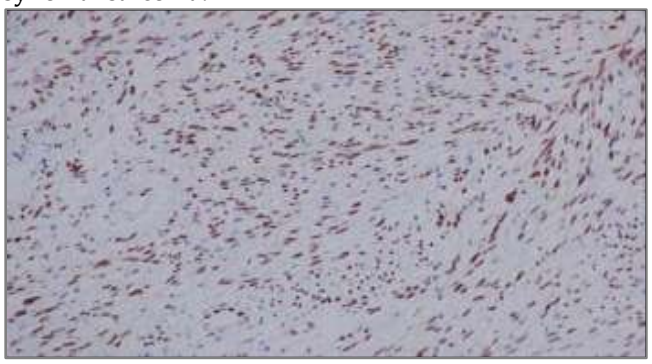

Figure-7: Mild to moderate staining (1+) TLE-1 in a monophasic synovial sarcoma.

37 years and range was 20-73 years, both findings consistent with our results[10]. In a locoregional study conducted in India on 42 cases, the mean age at diagnosis stood at 25 years and age of patients ranged between 2-60 years[11]. Similarly another study in Brazil described the mean age of 35 years with a range of 5-81 years[7].

The male to female ratio in our study was 1.2:1 with 53% males and 47% female patients, hence no gender predilection was observed. A study conducted in Mexico described the tumor in 53% female and 47% males in a study of 15 cases of pleuropulmonary SS[10]. Similarly no gender predisposition was seen in a study carried out in India which described male to female ratio as 1:1[11]. A multicenter study in Sweden and Italy found 54% of patients were males and 46% females in a study of 121 cases of SS[1]. Male to female ratio in study conducted in a study at Brazil was 1.06:1[7].

In our study, 76% of tumor (n=46) were found in extremities around tendon sheaths, a finding in concordance with a study in India which described 63% of primary SS in extremities and a study from Brazil which found 78% of cases in upper and lower limbs[13,14].

An Egyptian study concluded that 71 (96%)

Table: IHC staining results for synovial sarcoma.

Tumor type	Scoring			*p*-value
	No Staining	Mild Staining (1+)	Diffuse Staining (2+)	
Mono-phasic SS*	2	18	22	0.023
Biphasic SS*	4	1	10	
Poorly Differen-tiated SS*	-	2	1	

*Synovial Sarcoma

of 74 synovial sarcomas were positive for TLE1, including 37 biphasic (95%) and 34 monophasic (97%) tumors. It was concluded that overall sensitivity and specificity of TLE1 expression for the diagnosis of SS were 96% and 85% respectively[3].

A study conducted in Malaysia described nuclear immunoreactivity in 22 of 26 (84.6%) SS cases, including 11 of 12 (91.7%) biphasic type, 10 of 12 (83.3%) monophasic type and 1 of 2 (50%) poorly differentiated type. None of the non-SS cases showed strong nuclear positivity (score 3+)[9]. A study conducted in Germany demonstrated that TLE-1 is expressed in 96% of cases of SS in a study of 259 cases[15]. A study from Houston America described observed diffuse positivity of TLE-1 in 60% of the cases of SS[16]. Similarly another study of 35 (100%) all cases of SS concluded TLE-1 being immunoreactive to TLE-1[5]. Out of 26 cases in Malaysia found 84% of cases

positive for TLE-1[9]. Another study carried out by Xin *et al*, described immunoreactivity for TLE-1 in 94% of cases[18]. All these results were similar to findings observed in our study.

In our study 40 (95%) cases of monophasic SS were positive for TLE-1 and 11 (73%) cases of biphasic synovial sarcomas were positive for TLE-1. All 3 cases of poorly differentiated SS showed immunoreactivity to TLE-1. A study from Egypt described 95% biphasic SS and 97% of mono-phasic SS being immunoreactive to TLE-1[3]. Similarly another study concluded by Jgadis *et al* found 96% of monophasic and 100% of biphasic, poorly differentiated SS positive for TLE-1[5]. A study in Malaysia described 83% of monophasic, 91% of biphasic and 50% of poorly differentiated SS being immunoreactive to TLE-1[9].

On the contrary, Kosemehmetoglu *et al*, stated that TLE1 expression is not specific for synovial sarcoma as and it is also expressed in other tumor included in differentials diagnosis of SS, particularly tumors of peripheral nerve sheath origin. They also added that TLE1 is of value in diagnosing SS but it should be used in conjunction with other panel of antibodies to rule out other important mimickers[17-20].

CONCLUSION

TLE-1 was a sensitive and specific marker when SS is kept in differential of spindle cell soft tissue sarcomas. However, it should be interpreted with caution in combination with other immunohistochemical markers for dual confirmation wherever the diagnostic challenge occurs due to any reason. The gold standard for diagnosis, however, remains molecular testing of translocation t (X;18) that produces the fusion oncogenes SYT-SSX by FISH, when available.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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