Advanced Medical Journal, Vol.9, No.3, P.151-157,2024 https://doi.org/10.56056/amj.2024.288

TLE1 Expression in Different Sarcoma Types and its Relation to Clinicopathological Parameters

Toqa Jabbar Chckaim* Saman Salah Eldeen Abdulla Savan Saeed Azeez*****

Abstract:

Background and objectives: Split genes that contain Transducin-Like Enhancer encode transcriptional corepressors that are essential for hematopoiesis and embryogenesis in humans. Synovial sarcomas express Transducin-Like Enhancer-1, a distinct factor which hardly has been shown in other soft tissues malignancies. Transducin-Like Enhancer-1 expression was recently found to be sensitive but not entirely specific for synovial sarcoma.

Methods: This retrospective study included eighty paraffin embedded formalin-fixed blocks of different sarcomas which were gathered and picked out of the files of Rizgary teaching hospital lab and from certain private labs in Hawler, during June 2015-December 2022. In this study, Transducin-Like Enhancer-1, a mouse monoclonal antibody was applied on sarcoma cases and its expression was assessed.

Results: around one third (30%) of the patients had synovial sarcoma, (16.3%) had undifferentiated pleomorphic sarcoma, and the rest had other types. More than half (55%) of the sarcomas were of high grade, and the Transducin-Like Enhancer-1 score was strongly reactive in 33.8% of the patients. It was also strong in all (100%) the patients with synovial sarcoma, compared with 23.1% of patients with undifferentiated pleomorphic sarcoma, and 0% of the other types of sarcoma ($p < 0.001$).

Conclusion: this study demonstrates that, when synovial sarcoma was kept in the differential diagnosis of spindle cell soft tissue sarcomas, Transducin-Like Enhancer-1 was a highly sensitive and relatively a specific marker.

Keywords: Immunohistochemistry, Synovial sarcoma, TLE1.

*MBChB, MSc.Path, SHO of Histopathology, KBMS trainee in Rizgary Teaching Hospital, Erbil.
**MBChB, FRC.Path, RCPI, Ass.Prof. Department of Basic Sciences/Pathology, College of Dentistry, Hawler Medical University, Erbil. samhisto@gmail.com
***MBChB, FKCM, Path, Lecturer, Department of Anatomy and Pathology/Pathology, College of Medicine, Hawler Medical University, Erbil. savan.shengola@hmu.edu.krd
Author corresponding: Toqa Jabbar Chckaim. Email: toqa58refaee@gmail.com

Introduction

Sarcomas, malignancies with a presumed mesenchymal origin, make up fewer than 1% of solid malignant tumors in adults and over 21% of solid malignant tumors in children.[1] Incidence rate of sarcomas is the same in Iraq and in Kurdistan.[2]Typically, benign soft tissue tumors do not dedifferentiate into soft tissue sarcomas; rather, they form de novo. Although the cause of soft tissue sarcomas is unknown, there are some factors that are linked to a greater risk, such as environmental and genetic influences (like radiation, viral infections (HHV-8), chemical carcinogens, immune system deficiencies).[3]The heterogeneous category of sarcomas known as soft tissue sarcomas has distinctive histologic characteristics. Unfortunately, many sarcomas cannot be accurately diagnosed using histology alone. Immunohistochemistry (IHC) is crucial in these situations for pinpointing the line of differentiation and precise characterization.[4]In soft tissue tumors, immunohistochemical (IHC) staining is used to look for the presence of antigens specific to a certain lineage. Following a detailed evaluation of morphological aspects, IHC staining is done, opening the door to potential differential diagnosis. The initial antibody panel identifies the broad lineage, while subsequent panels clarify the type of tumor.[5]Split genes that contain Transducin-Like Enhancer (TLE) encode transcriptional corepressors that are essential for hematopoiesis and embryogenesis in humans.[6]There are 19 exons in the Transducin-like enhancer of split 1 (TLE 1) gene, which is one of four genes in the family and is found on chromosome 9q21. TLE1 protein interacts with numerous proteins to create homo- and hetero-oligomers. Once bound, it inhibits transcriptional activity, particularly in the Wnt signaling pathway where it interacts with catenin and T-cell factor.[7]Synovial sarcomas express TLE1, a distinct factor which hardly has been shown in other soft tissues malignancies. Additionally, it was shown wherein TLE1 is only seen in synovial sarcomas and not in other healthy stromal tissues, indicating TLE1 may be a target for treatment.[8]A commercially available antibody for TLE1 was sensitive and specific in separating synovial sarcoma from other soft tissue malignancies, according to Terry et al. retrospective's investigation of soft tissue tumor microarrays.[9] Transducin-Like Enhancer-1 expression was recently found to be sensitive but not specific for synovial sarcoma, according to a whole section investigation by Kosemehmetoglu et al.[10] This study aimed to assess TLE1 expression in different types of sarcoma by using immunohistochemistry, in addition to investigate its association with some clinico-pathologic parameters, such as the age, gender of patients, as well as tumor type (sarcomas that are not synovial and those that are) and tumor grade.

Material and methods:

This retrospective study included eighty paraffin embedded formalin-fixed blocks of soft tissue specimens diagnosed as different types of sarcoma which were selected randomly from the files of Rizgary teaching hospital lab and from certain private labs in Hawler, during June 2015-December 2022. The soft tissue sarcoma specimens that were included in this study were as: 24 synovial sarcoma cases, 13 undifferentiated pleomorphic sarcoma cases, 7 liposarcoma cases, 7 fibrosarcoma cases, 6 chondrosarcoma cases, 6 Ewing sarcoma cases, 6 DFSP (Dermatofibrosarcoma protuberans) cases, 4 rhabdomyosarcoma cases, 3 osteosarcoma cases, 2 leiomyosarcoma cases, a case of angiosarcoma and a case of spindle cell sarcoma.Two sections were prepared from each block, one stained with Hematoxylin & Eosin for the purpose of histological analysis,

https://doi.org/10.56056/amj.2024.288 https://amj.khcms.edu.krd

while the other was used for immunohistochemical evaluation of TLE1 expression. The microscopic classification & grading of soft tissue sarcoma cases were performed according to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) system. Ethical approval was obtained from the Ethics Committee of Kurdistan Higher Council of Medical Specialties.Immunohistochemical method: Four μm thick sections were cut, put on charged slides. After drying a single hour at 60 °C, slides were deparaffinized then rehydrated at 20 to 25 °C room temperature. They were submerged in xylene and five minutes' later incubation, they spent the next 3 minutes in ethanol. Lastly, immersion in distilled water was done for 30 seconds. Epitope retrieval was carried out by using a specific method in 10 mmol/L citrate buffer 1:10 ratio with distilled water. Immunohistochemical staining was performed using (DakoEnVision FLEX+) system. TLE1 antibody, a mouse monoclonal antibody (Clone: 1F5; catalog no: BSB 2318; 0.5ml concentrated; dilution 1:200) was applied on tissue sections. The reactivity for TLE1 was considered as positive when nuclear staining was observed, and scoring of TLE1 immunoreactivity was completed and examined by two professional pathologists. Positive and negative control slides were involved with each run of staining. By leaving out the main antibody and utilizing the N-Universal negative control, negative controls were created, and epithelial and endothelial cell staining were used as positive control for TLE1 expression.Scoring system: The stained slides were inspected under a light microscope for IHC staining analysis to determine the proportion and degree of staining in accordance with the Remmele score (0–12): Remmele score = intensity of immunoreactivity × percentage of the stained tumor cells. The Remmele Score measured the intensity of immunoreactivity as follows: zero for no staining, one for a weak stain (faint light-brown staining), two for a moderate/intermediate stain (dark-brown nuclear staining of intensity lower than that of positive control), and three for a strong stain (dark-brown nuclear staining of intensity comparable to that of positive control). The percentage of positively stained cells was calculated as follows: zero for no staining, one for less than 10% of the cells, two for more than 50% of the cells, three for more than 80% of the cells, and four for 81-100% of the cells staining. The entire score was translated into the following ranges: 5 to 12 = high, 3 to 4 = moderate, 1 to 2 = weak, and 0 = negative. High and moderate scores were considered as positive TLE1 staining, whereas weak score was considered negative.[4,5]Statistical analysis: Results were analyzed using Statistical Package for Social Sciences (SPSS, version 26). Chi square test of association was used to compare proportions of two or more groups. Fisher's exact test was used when the expected frequency (value) was 5 or fewer of more than 20% of the cells of the table. A p value of $\leq$ 0.05 was regarded as statistically significant.

Results:

The study involved 80 patients, with a median age of 41 years and a mean age (SD) of 61.6 (21.6) years. The range of ages was 2 to 85. 75% of the patients were over 20 years old, and 57.5% of them were male Table (1).

https://doi.org/10.56056/amj.2024.288 https://amj.khcms.edu.krd

Table (1): Age and gender distribution.

	No.	%
Age (years)		
≤ 20	20	25.0
> 20	60	75.0
Gender		
Male	46	57.5
Female	34	42.5
Total	80	100.0

30% of the patients had synovial sarcoma, of which 75% had monophasic and 25% had biphasic synovial sarcoma; 16.3% had undifferentiated pleomorphic sarcoma; and the remaining had various forms of sarcoma as follows: seven cases each of liposarcoma, fibrosarcoma; six cases each of chondrosarcoma, Ewing sarcoma, DFSP; four cases of rhabdomyosarcoma; three cases of osteosarcoma; two cases of leiomyosarcoma; a case each of angiosarcoma, and spindle cell sarcoma.More than half (55%) of the sarcomas were of high grade, and the TLE1 score was strongly reactive in 33.8% of the patients Table (2).

Table (2): Sarcoma characteristics.

	No.	%
Type		
Synovial sarcoma	24	30.0
Other sarcomas	43	53.8
Undifferentiated pleomorphic sarcoma	13	16.3
Grade		
Low grade	20	25.0
Intermediate grade	16	20.0
High grade	44	55.0
TLE1 score		
Negative	52	65.0
Moderate reactivity	1	1.3
Strong reactivity	27	33.8
Total	80	100.0

The TLE1 immuno-reactivity was strong in 50% of the females, compared with 21.7% of males ($p = 0.006$). It was also strong in all (100%) cases of synovial sarcoma (regardless of histologic grade and type), when compared to 23.1% of patients with undifferentiated pleomorphic sarcoma, and 0% of the other types of sarcoma ($p < 0.001$). There were no differences between the histologic categories of synovial sarcoma in the prevalence of TLE1 positivity, also both the spindle and epithelial cell components showed TLE1 positivity. No significant association was detected between TLE1 immuno-reactivity with age ($p = 0.692$) and grade ($p = 0.714$) Table (3).

https://doi.org/10.56056/amj.2024.288 https://amj.khcms.edu.krd

Table (3): TLE1 immunoreactivity according to age, gender, sarcoma type and grade.

		TLE1 immuno-reactivity			
		Negative	Moderate	Strong	
	N	No. (%)	No. (%)	No. (%)	P*
Age					
≤ 20	20	12 (60.0)	0 (0.0)	8 (40.0)	
> 20	60	40 (66.7)	1 (1.7)	19 (31.7)	0.692
Gender					
Male	46	36 (78.3)	0 (0.0)	10 (21.7)	
Female	34	16 (47.1)	1 (2.9)	17 (50.0)	0.006
Type					
Synovial sarcoma	24	0 (0.0)	0 (0.0)	24 (100.0)	
Other sarcomas	43	43 (100.0)	0 (0.0)	0 (0.0)	
Undifferentiated pleomorphic sarcoma	13	9 (69.2)	1 (7.7)	3 (23.1)	< 0.001
Grade					
Low grade	20	11 (55.0)	0 (0.0)	9 (45.0)	
Intermediate grade	16	11 (68.8)	0 (0.0)	5 (31.3)	
High grade	44	30 (68.2)	1 (2.3)	13 (29.5)	0.714
Total	80	52 (65.0)	1 (1.3)	27 (33.8)	

*By Fisher's exact test.

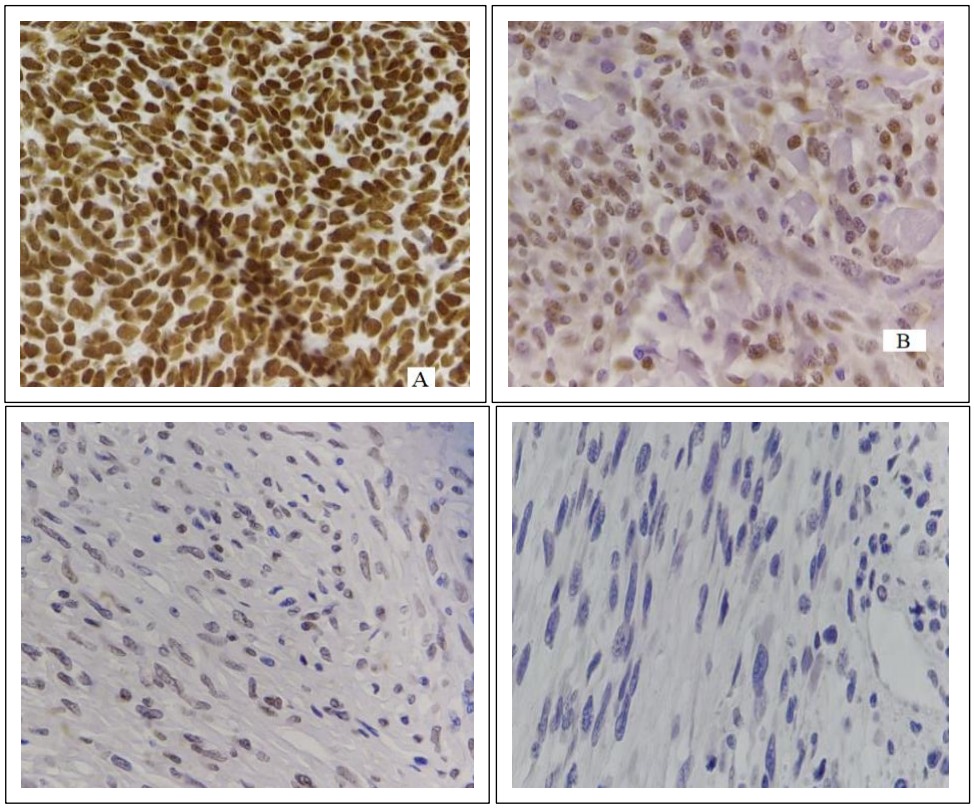

Figure (1): TLE1 immunoexpression. A. Strong TLE1 expression (IHCx400). B. Moderate TLE1 expression (IHCx400). C. Weak TLE1 expression (IHCx400). D. Negative TLE1 expression (IHCx400).

https://doi.org/10.56056/amj.2024.288 https://amj.khcms.edu.krd

Discussion:

TLE1 has become a very precise and sensitive marker for separating synovial sarcoma from its imitators.[4] Human transcriptional corepressors involved in hematopoiesis and embryogenesis are encoded by the TLE1 genes. TLE1 was shown to be overexpressed in the nucleus of synovial sarcoma cells, according to gene expression analyses.[4] This study assessed TLE1 expression in synovial and non-synovial sarcomas. The two markers that we depended on in our investigation for the identification of synovial sarcoma were TLE1 and EMA. TLE1 displayed extensive nuclear immunoreactivity in a significant fraction of cells, in contrast to the localized staining pattern of EMA in synovial sarcoma. Contrary to Knosel et al observation of 96% positive TLE1 expression,[11] This study revealed that all synovial sarcoma patients have TLE1 expression, exactly the exact same proportion discovered by Qureshi et al.[4] Our study discovered TLE1 immunostain as an incredibly sensitive but non-specific marker for synovial sarcoma. In this study TLE1 was strongly positive in 24 out of 24 (100%) of synovial sarcoma cases, while it was moderately and strongly positive in 4 of 13 (38%) of undifferentiated pleomorphic sarcomas. Comparable results were obtained by other studies, as Kosemehmetoglu et al. research, 53 of 143 (37%) non-synovial sarcomas had TLE1 expression.[10] TLE1 has been identified by Foo et al. as a sensitive and specific marker for synovial sarcoma that may be useful in separating it from histologic mimickers, especially if moderate or severe staining is seen.[12] Rekhi et al.'s study found that TLE1 was expressed in 95.2% of synovial sarcomas,[13] Zafar Ali et al. discovered that it was expressed in 24 out of 25 synovial sarcomas,[14] by Atef et al. 96%,[15] Bakrin IH et al.'s 84.6%, [16] 100% by Jagdis A and colleagues, [17] and by Xin et al. 94%.[18] This discrepancy in results can be explained by the difference in sample size, variable antibody sources, different properties of antibodies and scoring system.Transducin-like enhancer of split-1 expression is not only present in synovial sarcomas, according to Kosemehmetoglu et al.; other tumors which are considered synovial sarcoma's differential diagnosis, also express TLE1 like, neurofibroma, schwannoma, and malignant peripheral nerve sheath tumor.[10] Transducin-like enhancer of split-1 is useful in the diagnosis of synovial sarcoma, yet it must be utilized in concert a panel of additional antibodies to rule out further significant mimickers. [10]Based on our data, TLE1 is more likely to serve as an accurate biomarker for synovial sarcoma. TLE1's expression by IHC will help rule out or confirm the diagnosis of synovial sarcoma due to its high sensitivity and specificity. TLE1 is often overexpressed in synovial sarcomas, the vast majority of studies show, including our own. Additionally, Seo et al.[8] described that TLE1 may be a possible treatment target for synovial sarcomas because it has been shown that TLE1 is essential for the survival of synovial sarcoma cells and that normal cells with mesenchymal ancestry never express TLE1.

Conclusion:

This study indicates that TLE-1 was a very sensitive and somewhat specific marker when synovial sarcoma was retained in the differential diagnosis of spindle cell soft tissue sarcomas. Wherever the diagnostic challenge arises for any reason, it should be evaluated cautiously in conjunction with other immunohistochemical markers for dual confirmation. Nonetheless, when accessible, the gold standard for the diagnosis of synovial sarcoma remains molecular testing of the translocation t (X;18) that gives rise to the fusion oncogenes SYT-SSX.

Conflict of interest

The author reports no conflicts of interest.

https://doi.org/10.56056/amj.2024.288 https://amj.khcms.edu.krd

References:

1.Burningham Z, Hashibe M, Spector L, Schiffman Joshua D . The epidemiology of sarcoma. Clin. Sarcoma Res. 2012;2(1):1-16.
2.Hussain Ashraf MA, Lafta Riyadh K. Cancer trends in Iraq 2000–2016. Oman Med. J. 2021;36(1):e219.
3.van Vliet M, Kliffen M, Krestin G, van Dijke CF. Soft tissue sarcomas at a glance: clinical, histological, and MR imaging features of malignant extremity soft tissue tumors. Eur. Radiol. 2009;19:1499-511.
4.Qureshi MB, Uddin N, Tariq M, et al. Frequency of transducer-like enhancer of split 1 immunohistochemical expression in synovial sarcoma: an institution-based cross-sectional study. Cureus. 2019;11(12).
5.Qureshi MB, Tariq MU, Abdul-Ghafar J, Chundriger Q, Ud Din N. Diagnostic utility of TLE1 (transducer-like enhancer of split 1) in distinguishing synovial sarcoma from mimicking tumors. Int. J. Gen. Med. 2021:9173-9.
6.El Beaino M, Jupiter DC, Assi T, Rassy E, Lazar AJ, Araujo DM, et al. Diagnostic value of TLE1 in synovial sarcoma: a systematic review and meta-analysis. Sarcoma. 2020; 2020: 7192347.
7.Pinto K, Chetty R. Gene of the month: TLE 1. J. Clin. Pathol. 2021;74(3):137-40.
8.Seo SW, Lee H, Lee HI, Kim HS. The role of TLE1 in synovial sarcoma. J. Orthop. Res. 2011;29(7):1131-6.
9.Terry J, Saito T, Subramanian S, Ruttan C, Antonescu CR, Goldblum JR, et al. TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma emerging from gene expression profiling studies. Am. J. Surg. Pathol. 2007;31(2):240-6.
10.Kosemehmetoglu K, Vrana JA, Folpe AL. TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms. J. Mod. Hum. Pathol. 2009;22(7):872-8.
11.Knösel T, Heretsch S, Altendorf-Hofmann A, Richter P, Katenkamp K, Katenkamp D, et al. TLE1 is a robust diagnostic biomarker for synovial sarcomas and correlates with t (X; 18): analysis of 319 cases. Eur. J. Cancer. 2010;46(6):1170-6.
12.Foo WC, Cruise MW, Wick MR, Hornick JL. Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics. Am. J. Clin. Pathol. 2011;135(6):839-44.
13.Rekhi B, Basak R, Desai SB, Jambhekar NA. Immunohistochemical validation of TLE1, a novel marker, for synovial sarcomas. IJMR. 2012;136(5):766.
14.Ali Z, Khan AH, Rehman U, Faisal M, Ahmad IN, Mamoon N, et al. Is TLE1 expression limited to synovial sarcoma Our experience at shifa international hospital, Pakistan. Cureus. 2019;11(11).
15.Atef A, Alrashidy M. Transducer-like enhancer of split 1 as a novel immunohistochemical marker for diagnosis of synovial sarcoma. APJCP. 2015;16(15):6545-8.
16.Bakrin IH, Hussain FA, Sharif ST. Transducer-like enhancer of split 1 (TLE1) expression as a diagnostic immunohistochemical marker for synovial sarcoma and its association with morphological features. Malays. J. Pathol. 2016;38(2):117-22.
17.Jagdis A, Rubin BP, Tubbs RR, Pacheco M, Nielsen TO. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. Am. J. Surg. Pathol. 2009;33(12):1743-51.
18.He X, Xiong B, Zhou T, Lan T, Chen M, Chen H, et al. Diagnostic value of TLE1 for synovial sarcoma: immunohistochemical analyses of genetically confirmed synovial sarcomas and nonsynovial sarcomas. Int J Clin Exp Pathol. 2016;9(4):4339-50.