Role of NGS based genomic profiling analysis in identifying targetable genetic alterations in pediatric western Saudi Arabia sarcomas patients: Advanced insights into PTEN

Hassan Fouad Huwait[1], hfhuwait@uqu.edu.sa

Hanan Mohammed Abd Elmoneim[1,2*] hmnour@uqu.edu.sa

Mariana Fathy Gayyed[2], mariana.gaid@mu.edu.eg

Rabab Ahmed Moussa[2] , rabab.mohamed@mu.edu.eg

[1]Department of Pathology, Faculty of Medicine, Umm Al-Qura University, Makkah, Kingdom of Saudi Arabia.

[2]Department of Pathology, Faculty of Medicine, Minia University, Minia, Egypt.

Corresponding Author:

Rabab Ahmed Moussa

Email:

rabab.mohamed@mu.edu.eg

rababmossa00@gmail.com

Abstract

Background: PTEN is one of the frequently mutated genes in different sarcoma subtypes. This study aims to investigate the potential role and alterations of the PTEN gene in various pediatric sarcoma cases. Methods:169 Saudi pediatric sarcomas patients had full clinical and follow-up data. Next generation sequencing (NGS)-based genomic profiling analysis was done for pediatric sarcomas cases, including rhabdomyosarcoma (RMS), osteosarcoma (OS), and Ewing's sarcoma (ES). Detection of PTEN gene mutation and its asocaiation with loss of PTEN protein expression was investigated. Results: PTEN protein expression was lost in 54 (31.9%) of studied cases. For different sarcoma subgroups,

PTEN protein loss was correlated with diminished overall survival (P=0.04, P<0.001, and P=0.02, respectively). The commonly detected mutated genes in all samples were P53 (35%), PTEN (20.3%), and PIK3CA (18.5%). In some cases, PTEN gene mutation are presented with other mutated genes, including PTEN/p53 double mutation (7/11), and PTEN/PIK3CA coexistent mutation (3/11). PTEN gene mutation was detected in only 11 out of 54 cases with exhibted PTEN protein loss. Conclusion: absence of a correlation between PTEN protein loss and PTEN gene mutation may be associated with a frequency of other actionable mutations that can play an essential role in the tumorigenesis process.

Keywords: Ewing's sarcoma, next-generation sequencing , osteosarcoma, PTEN, rhabdomyosarcoma

INTRODUCTION

Sarcomas are rare neoplasms that originate from mesenchymal cells and can affect either soft tissue or bones. The most prevalent subtypes of primary bone sarcomas in Saudi Arabia are OS, ES, and chondrosarcoma (ChS) [1]. The incidence of sarcomas in pediatric patients is over 20%, whereas it is less than 1% in adults. The vast majority of sarcoma diagnoses are soft tissue sarcomas, while bone tumors account for slightly more than 10% of diagnoses [2]. RMS, a high-grade neoplasm of myoblast-like skeletal cells, is more prevalent in children than any other type of soft tissue sarcoma [3]. Sarcomas can be managed with innovative therapies that interfere with oncogenic mutations and altered pathways[4].

Phosphatase and tensin homolog (PTEN), a known tumor suppressor, has an ATP-binding transcriptional silencing and protein stability. PTEN Mutations, deletions, transcriptional silencing, and protein instability are common pathways that is repeatedly affecting various sarcoma histotypes. Some soft tissue sarcomas can also be characterized by the interaction of

PTEN with other tumor suppressors and oncogenic signaling pathways [5]. PTEN, the second most common tumor suppressor gene, is most commonly mutated in cancer patients, and its inactivation is responsible for developing several cancer types in humans, including glioblastoma, uterine, prostate, colorectal, and breast tumors. In some of these tumors, loss of PTEN expression is associated with the progression of tumor stages and resistance to targeted treatments, especially those targeting receptor tyrosine kinase pathways [6-8].

In this study, we have characterized PTEN on paraffin-embedded tissue from a diverse cohort of pediatric sarcomas using a comprehensive molecular and IHC approach including RMS, OS, and ES to determine the prevalence of PTEN expression and its correlation with the clinicopathological features of each tumor. Using NGS-based genomic panels, we also investigated the potential role and alterations of the PTEN gene in pediatric sarcoma cases.

MATERIALS AND METHODS

Tumor samples

Archival formalin fixed-paraffin embedded blocks were obtained from 169 patients with RMS, OS, and ES. Blocks were retrieved from the pathology files for patients diagnosed between December 2011 and January 2021. In addition, the Institutional Review Board approved the study procedure of Umm Al-Qura University. Data follow-up began at diagnosis, and the median follow-up was 60 months. Overall survival was defined as the time to death due to disease-related causes, and all archival blocks admitted for the first diagnosis were selected. Exclusion criteria included the archival blocks belonging to patients with incomplete clinical or missed follow-up data. Clinical records include age, gender, type of tumor, date of presentation of illness, history of clinical illness, tumor size, tumor localization, treatment regimen, and patient response rate at the

end of the study. All H&E-stained slides were reviewed from all cases to determine the sarcoma's histological diagnoses and subtypes according to the World Health Organization classification [9].

Immunohistochemical Analysis of PTEN

For IHC staining, formalin-fixed paraffin-embedded blocks were utilized. The paraffin-embedded material was cut into 4 μm sections, dewaxed with xylene, and rehydrated with graded ethanol. Afterward, the sections were microwave-irradiated in EDTA buffer for antigen retrieval. After endogenous peroxidase inhibition, sections were subjected to the primary antibody at 4°C overnight and stained with a streptavidin-biotin-peroxidase kit. Finally, sections immersed in 3.3′-diaminobenzidine were counterstained and mounted. In accordance with the manufacturer's guidelines for the PTEN detection system, IHC staining of paraffin block sections for PTEN (PTEN pharm Dx kit, DakoCytomation, Glostrup, Denmark) was performed. The anti-PTEN monoclonal antibody, clone 6H2.1, diluted 1/1000. For each run, negative controls were also included without the primary antibody. As positive controls for anti-PTEN antibodies, melanoma was utilized.

Based on the strength of staining and the proportion of positive cells, the right immunohistochemistry score of a tumor was determined. Two pathologists performed a semi-quantitative study, resulting in a scoring system that is calculated by multiplying the intensity by the percentage of expression. The positivity of PTEN staining was primarily detected in the cell cytoplasm, although it was also present in the nucleus. Negative or PTEN loss was defined as a score of less than 25% [10].

Next generation sequencing (NGS)

Genomic profiling using NGS was conducted on 54 samples of sarcoma. For DNA extraction from 4X10 m formalin-fixed paraffin-embedded sections, the conventional phenol/chloroform

extraction procedure was used. Until utilized as a template for NGS, genomic DNA was kept at -20^{o}C. A Qubit fluorometer was used to measure DNA quantity, and 20 ng of optimized DNA was utilized for sequencing. Hybrid-capture–selected libraries were sequenced to high uniform depth using the Illumina HiSeq2000 platform (Illumina, San Diego, CA, USA). Modifications were made to protocols and reagents to ensure uniform coverage and reliable results across a broad range of specimens. Base substitutions, indels, focal gene amplifications, homozygous gene deletions, and selective gene fusions were detected utilizing a tailored analytic workflow designed to accurately detect multiple types of genomic changes [11]. TruSight Tumor15 was used to evaluate gene performance. Qubit, a highly sensitive dsDNA assay, was utilized to test the value of the pooled libraries. On the MiSeq Platform, sequencing was performed using paired-end reads. After evaluating and comparing all reads with the hg19/GRCH37 reference sequence, the MiSeq reporter software was utilized to examine further the DNA sequence data. Base substitutions, insertions/deletions, copy number modifications, and rearrangements were all detected as genomic alterations.

Statistical Methods

Analyzing the data was done using the Statistical Package for Social Sciences (SPSS: An IBM Company, Version 21.0, IBM Corporation, Armonk, NY, USA). The chi-square test was performed to assess the relationship between PTEN expression and clinicopathological parameters as well as gene mutations. Kaplan-Meier plots and log-rank tests were used to evaluate the association between PTEN overexpression and overall survival. The level of statistical significance was determined at a p-value < 0.05.

RESULTS

Patients' demographics and clinicopathologic findings

The clinicopathological characteristics of the 169 participants in this study were derived from medical data and summarized in Table 1. The research group had 48 cases of RMS, 64 cases of OS, and 57 cases of ES.

Table 1: Clinico-pathological characteristics of 169 patients with paediatric sarcomas:

Clinico-pathological Characteristics		Rhabdomyosarcoma		Osteosarcoma		Ewing's sarcoma	
		No.	%	No.	%	No.	%
Age	0-10	27	56.3	29	45.3	17	29.8
	10-19	21	43.8	35	54.7	40	70.2
Gender	Male	33	68.8	38	59.4	39	68.4
	Female	15	31.3	26	40.6	18	31.6
Site	Trunk	22	45.8	39	60.9	29	50.9
	Extremities	26	54.2	25	39.1	28	49.1
Size	< 5 cm	21	43.8	38	59.4	31	54.4
	≥ 5 cm	27	56.3	26	40.6	26	45.6
Depth	Superficial	22	45.8	29	45.3	26	45.6
	Deep	26	54.2	35	54.7	31	54.4
Grade	Grade 1	12	25.0	19	29.7	11	19.3
	Grade 2	17	35.4	17	26.6	21	36.8
	Grade 3	19	39.6	28	43.8	25	43.9
Stage	Stage 1	9	18.8	13	20.3	10	17.5
	Stage 2	11	22.9	18	28.1	13	22.8
	Stage 3	28	58.3	33	51.6	34	59.6
Vascular invasion	Absent	35	72.9	51	79.7	35	61.4
	Present	13	27.1	13	20.3	22	38.6
Metastasis	Absent	30	62.5	42	65.6	27	47.4

	Present	18	37.5	22	34.4	30	52.6
Survival	Survival	30	62.5	49	76.6	39	68.4
	Death	18	37.5	15	23.4	18	31.6
Recurrence	Absent	28	58.3	43	67.2	29	50.9
	Present	20	41.7	21	32.8	28	49.1
PTEN	Loss	17	35.4	20	31.3	17	29.8
	Normal	31	64.6	44	68.8	40	70.2

Correlation between PTEN protein expression and clinicopathological data in pediatric sarcoma cases

According to our findings, PTEN protein expression was lost in 54 (31.9%) of the 169 sarcoma patients. PTEN protein staining pattern among different sarcoma cases is depicted in Figure 1 (A-F).

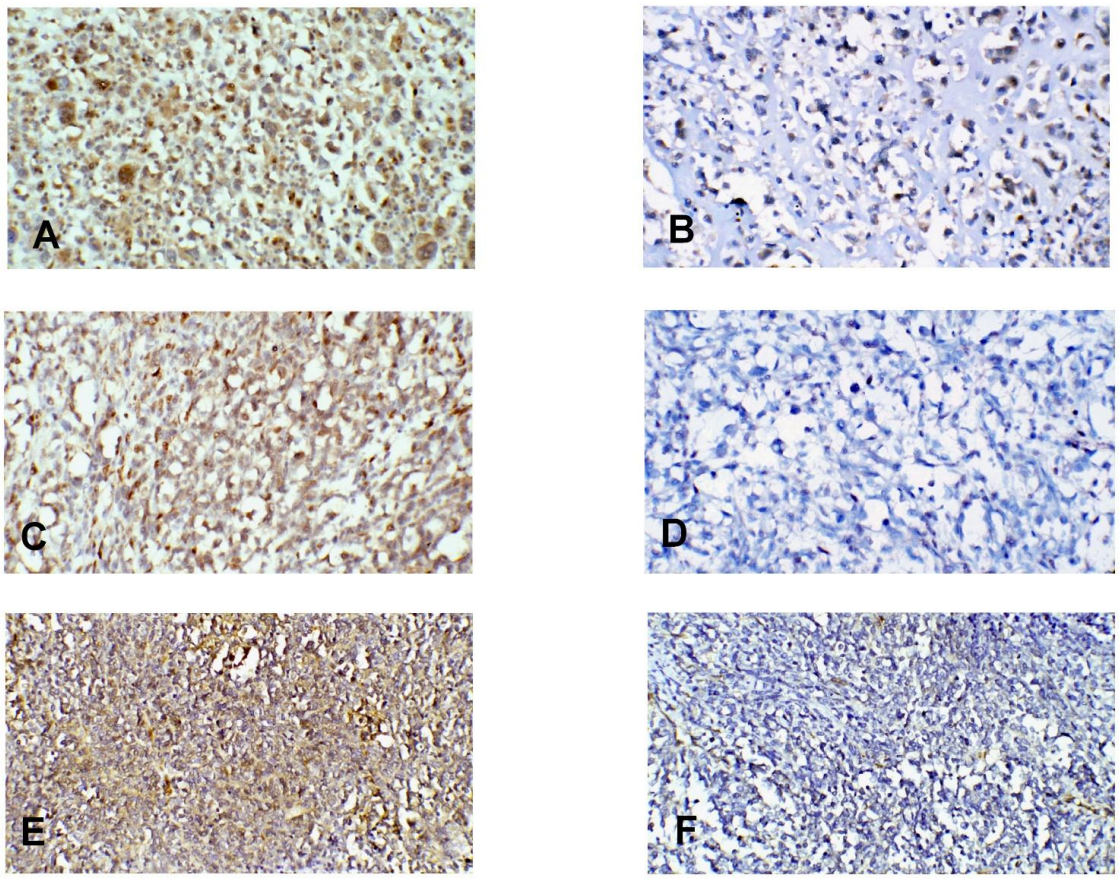

Figure 1. different Immunohistochemical staining patterns of PTEN in pediatric sarcoma samples. Normal immunohistochemical staining in osteosarcoma, rhabdomyosarcoma and Ewing's sarcoma cases. (A, C and E respectively, x 200). lost PTEN immuno-expression in osteosarcoma, rhabdomyosarcoma and Ewing's sarcoma cases (B, D and F respectively, x200).

Regarding correlation of PTEN protein expression with various clinicopathological data, PTEN protein loss was associated with higher histological grade, advanced tumor stage, vascular invasion, metastasis, and poor survival in RMS cases. In OS cases, loss of PTEN was significantly associated with advanced stage, metastasis, and poor survival. Meanwhile, the absence of PTEN protein was significantly related to advanced stages, metastasis, recurrence and poor survival in ES cases. On the contrary, in various sarcoma types, there was no significant relationship between PTEN expression and age, gender, tumor size, or initial tumor site (Table 2).

Table 2. Association of PTEN protein expression with clinicopathological features of different pediatric sarcoma cases:

Clinico-pathological Characteristics		Rhabdomyosarcoma			Osteosarcoma			Ewing' sarcoma		
		PTEN			PTEN			PTEN		
		Loss	Normal	P value	Loss	Normal	P value	Loss	Normal	P value
Age	0-10	10 (37.0%)	17 (63.0%)	0.7	11 (37.9%)	18 (62.1%)	0.3	7 (41.2%)	10 (58.8%)	0.2
	10-19	7 (33.3%)	14 (66.7%)		9 (25.7%)	26 (74.3%)		10 (25.0%)	30 (75.0%)	
Gender	Male	9 (27.3%)	24 (72.7%)	0.8	12 (31.6%)	26 (68.4%)	0.9	12 (30.8%)	27 (69.2%)	0.8
	Female	8 (53.3%)	7 (46.7%)		8 (30.8%)	18 (69.2%)		5 (27.8%)	13 (72.2%)	
Site	Trunk	7 (31.8%)	15 (68.2%)	0.6	10 (25.6%)	29 (74.4%)	0.2	10 (34.5%)	19 (65.5%)	0.4
	Extremities	10 (38.5%)	16 (61.5%)		10 (40.0%)	15 (60.0%)		7 (25.0%)	21 (75.0%)	
Size	< 5 cm	8 (38.1%)	13 (61.9%)	0.7	13 (34.2%)	25 (65.8%)	0.5	6 (21.4%)	22 (78.6%)	0.1
	≥ 5 cm	9 (33.3%)	18 (66.7%)		7 (26.9%)	19 (73.1%)		11 (37.9%)	18 (62.1%)	
Depth	Superficial	10 (45.5%)	12 (54.5%)	0.1	7 (24.1%)	22 (75.9%)	0.2	9 (34.6%)	17 (65.4%)	0.4
	Deep	7 (26.9%)	19 (73.1%)		13 (37.1%)	22 (62.9%)		8 (25.8%)	23 (74.2%)	
Grade	Grade 1	1 (8.3%)	11 (91.7%)		4 (21.1%)	15 (78.9%)		1 (9.1%)	10 (90.9%)	
	Grade 2	6 (35.3%)	11 (64.7%)	**0.01**	3 (17.6%)	14 (82.4%)	0.4	5 (23.8%)	16 (76.2%)	0.2
	Grade 3	10 (52.6%)	9 (47.4%)		13 (46.4%)	15 (53.6%)		11 (44.0%)	14 (56.0%)	
Stage	Stage 1	0 (0.0%)	9 (100.0%)		2 (15.4%)	11 (84.6%)		0 (0.0%)	10 (100.0%)	
	Stage 2	3 (27.3%)	8 (72.7%)	**0.004**	3 (16.7%)	15 (83.3%)	**0.01**	4 (30.8%)	9 (69.2%)	**0.02**
	Stage 3	14 (50.0%)	14 (50.0%)		15 (45.5%)	18 (54.5%)		13 (38.2%)	21 (61.8%)	
Vascular invasion	Absent	9 (25.7%)	26 (74.3%)	**0.02**	15 (29.4%)	36 (70.6%)	0.5	8 (22.9%)	27 (77.1%)	0.1
	Present	8 (61.5%)	5 (38.5%)		5 (38.5%)	8 (61.5%)		9 (40.9%)	13 (59.1%)	
Metastasis	Absent	6 (20.0%)	24 (80.0%)	**0.003**	9 (21.4%)	33 (78.6%)	**0.01**	5 (16.7%)	25 (83.3%)	**0.02**
	Present	11(61.1%)	7 (38.9%)		11 (50.0%)	11 (50.0%)		12 (44.4%)	15 (55.6%)	
Recurrence	Absent	11 (39.3%)	17 (60.7%)	0.5	12 (27.9%)	31 (72.1%)	0.4	5 (17.2%)	24 (82.8%)	**0.03**
	Present	6 (30.0%)	14 (70.0%)		8 (38.1%)	13 (61.9%)		12 (42.9%)	16 (57.1%)	
Survival	Survival	7 (23.3%)	23 (76.7%)	**0.02**	7 (14.3%)	42 (85.7%)	**0.001**	8 (20.5%)	31 (79.5%)	**0.02**
	Death	10 (55.6%)	8 (44.4%)		13 (86.7%)	2 (13.3%)		9 (50.0%)	9 (50.0%)	

Association of PTEN protein expression with Five -year overall survival (OS) rate

Poor overall survival was associated with a greater than 25% of tumor cells that had low or absent immunologic responses to PTEN in RMS, OS, and ES cases (P=0.04, P<0.001, and P=0.02, respectively) Figure 2 (A, B and C).

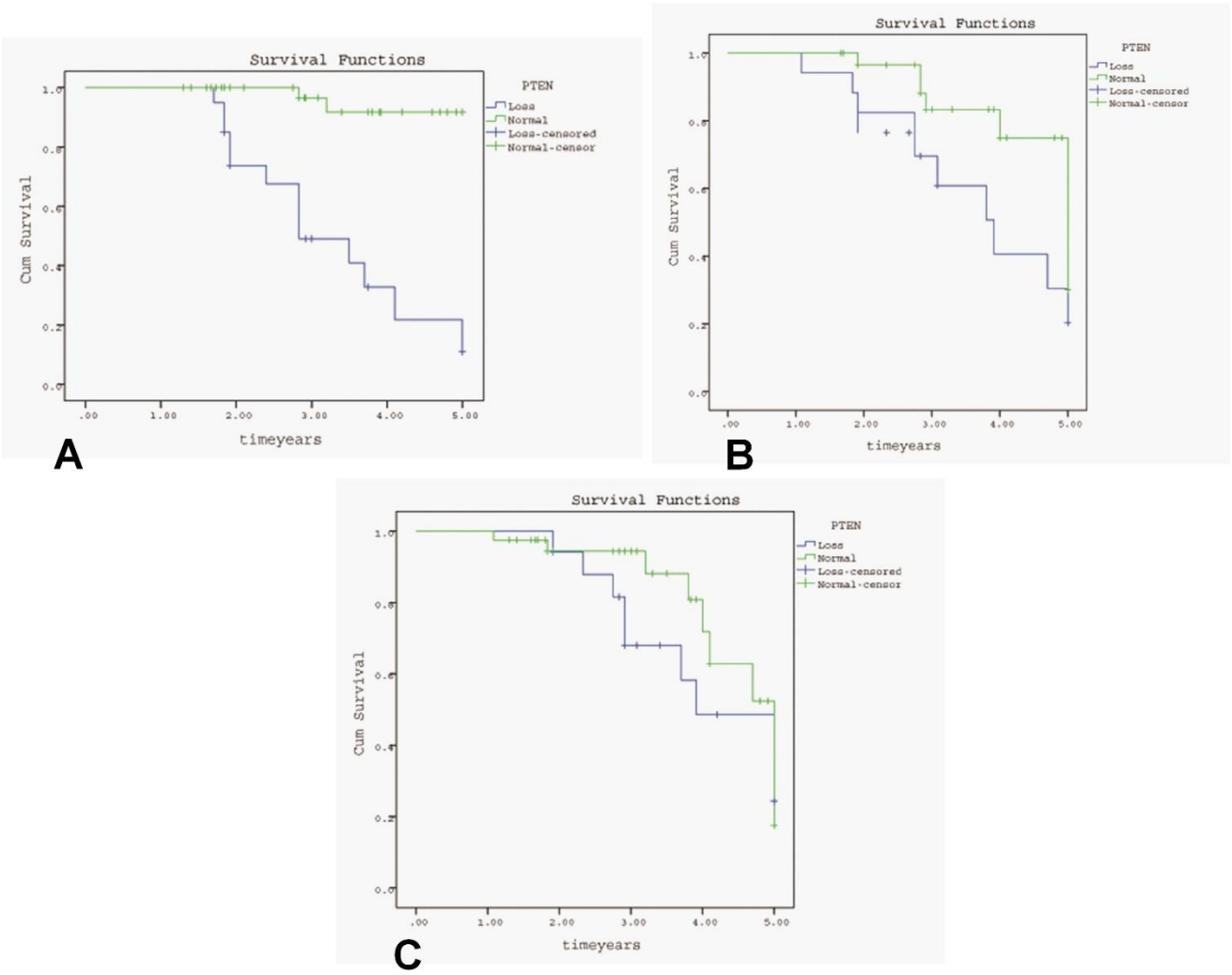

Figure 2.: Survival analysis according to cumulative Kaplan-Meier method in relation to PTEN protein expression in Rhabdomyosarcoma (A), Osteosarcoma (B) and Ewing's sarcoma (C) cases.

NGS-based genomic profiling

Fifty-four sarcoma samples that showed PTEN protein loss were selected for genomic profiling using NGS and different gene mutations were detected in each sarcoma group. RMS showed mutations in (FLT3, PIK3CA, PTEN, PTPN11, SMARCB1 genes). For the OS group, the detected gene mutations included (KIT, FLT3, TP53, MYC, PTEN, PDGFR, CDKN2A, BRAF, and RB1) while gene mutations in the ES group were (EWSR1-FLI1, APC, ATM, HNF1A, PTEN, CCND1) Figure 3 (A, B and C) respectively.

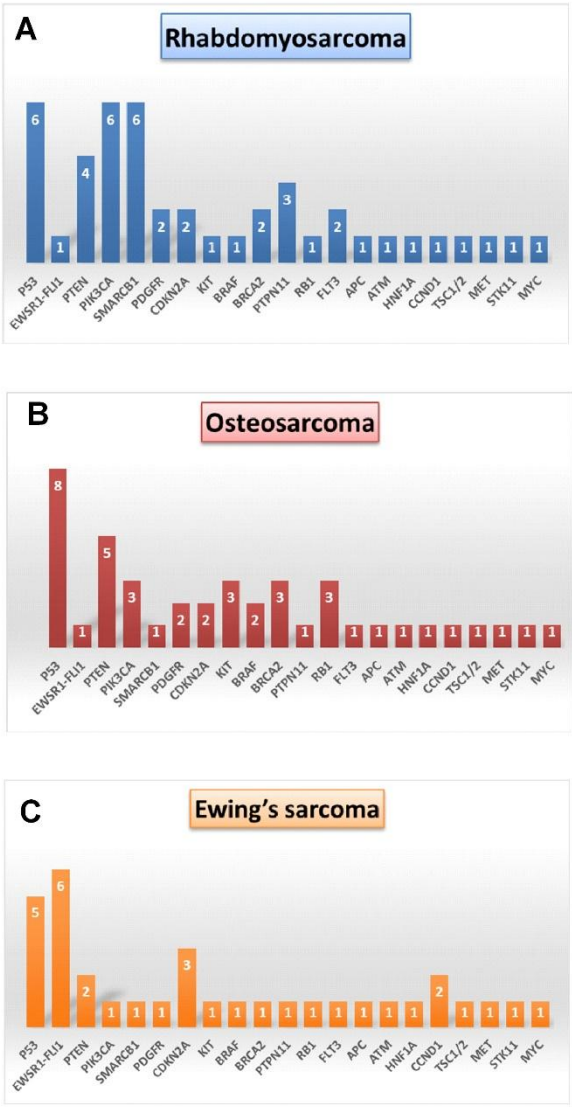

Figure 3 : Frequency of abnormal genes detected in A)Rhabdomyosarcoma, B) Osteosarcoma and C) Ewing's sarcoma cases.

Distribution of different mutational changes among selected cases with loss of PTEN protein expression:

In these three pediatric sarcomas, mutational analysis of the PTEN gene in relation to PTEN protein expression was performed. Regarding the distribution of different mutational changes in sarcoma cases with loss of PTEN protein expression, p53, PTEN, and PIK3CA were the most frequently detected mutant genes in all samples. PTEN gene mutational changes were detected in 20.4 % (11/54), while the distribution of other mutations was 35.2% (19/54) cases for P53 mutations and 18.5% (10/54) for PIK3CA mutations (Figure 4). Sarcoma cases demonstrated combined PTEN protein loss with normal PTEN gene status exhibited frequent mutational changes in both the p53 and PIK3CA genes, 27.9% (12/43) and 16.3% (7/43), respectively. Additionally, cases showed combined PTEN protein loss with PTEN gene mutation, with some exhibiting additional mutations, including p53 mutation (7/11), followed by PIK3CA mutation (3/11) (See Table 3).

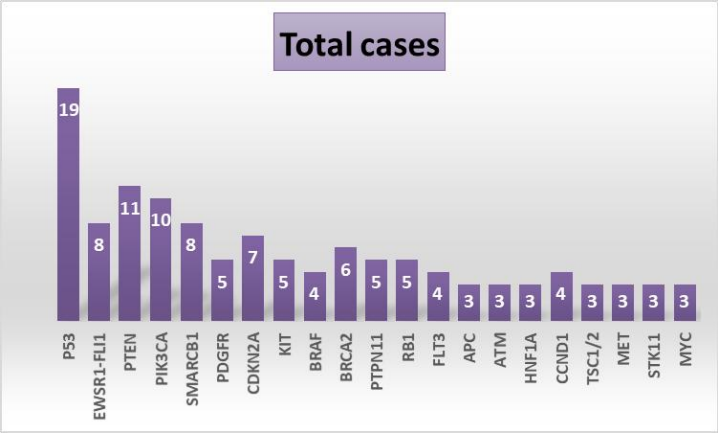

Figure 4. Frequency of different genes mutations detected in total sarcoma cases.

Table 3: Percentage of mutated p53 and PIK3CA genes among cases with normal and mutated PTEN gene status.

PTEN	p53		PIK3CA	
	Wild N (%)	Mutated N (%)	Normal N (%)	Mutated N (%)
Normal	31(72.1%)	12 (27.9%)	36 (83.7%)	7 (16.3%)
Mutated	4 (36.4%)	7 (63.6%)	8 (72.7%)	3 (27.3%)

Association between different mutational changes with overall survival in cases with PTEN protein loss expression*:*

For sarcoma cases with loss of PTEN protein expression, mutational changes in PTEN, PIK3CA, and p53 genes did not show an association with OS (P>0.05). In addition, there was no significant association with OS in relation to combined multiple different mutations (P>0.05), as depicted in Figure 5 (A-C) and Figure 6.

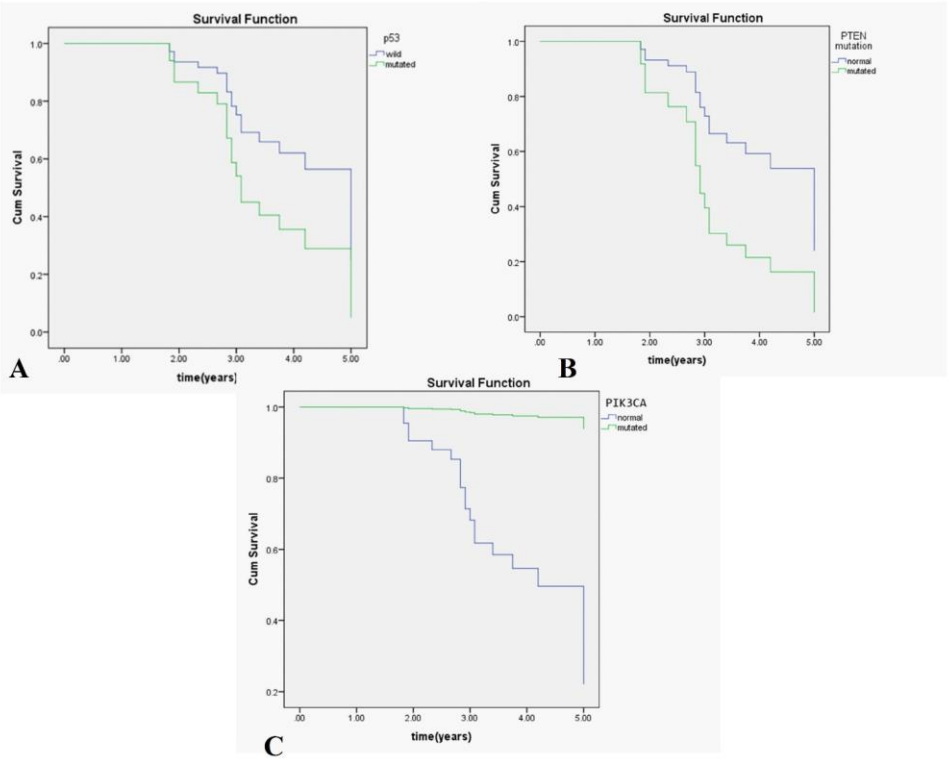

Figure 5. : survival analysis according to cumulative Kaplan-Meier method in relation to most frequent detected gene mutations in all different sarcoma cases, A) p53, B) PTEN and C) PIK3CA

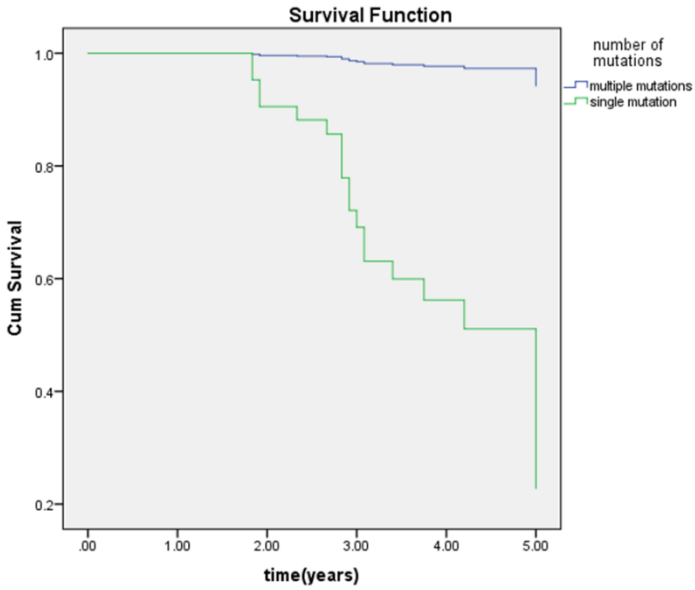

Figure 6: survival analysis according to cumulative Kaplan-Meier method in relation to multiple combined frequently detected genes mutations (p53, PTEN and PIK3CA) in all different sarcoma cases.

DISCUSSION

PTEN is a well-known tumor suppressor encrypting a protein of 403 amino acids, with phosphatase-dependent and phosphatase-independent activities. It is located on chromosome 10q23[5].

Over the past decades, the overall survival rate of pediatric sarcomas has hardly improved. The initial diagnosis of this type of tumor highly depends on immunohistochemistry. NGS analysis of sarcomas identified numerous somatic alterations with predictive or prognostic significance [12]. The discovery of a link between genetic changes and response to standard-of-care medicines opens the possibility of identifying novel prognostic and predictive biomarkers of treatment response, which

might contribute to better classification of patients with responder versus non-responder signatures [13].

In this study, loss of PTEN protein expression was found in 31.9% of all pediatric sarcoma cases, which is consistent with other results that demonstrated a comparable PTEN protein loss rate; 38.6% of sarcomas including epithelioid sarcoma, alveolar RMS, OS and 37.6% of uterine sarcoma [14].

Our results demonstrated that loss of PTEN protein expression was significantly associated with advanced stage, and the likelihood of metastasis in all three sarcomas (RMS, OS, and ES). These findings align with others who reported that PTEN loss is linked to advanced tumor stage and treatment resistance in gastrointestinal stromal tumors, particularly to therapies targeting tyrosine kinases receptors and related routes [10, 15]. In addition, we reported a significant association between the loss of PTEN protein expression and poor overall survival, which is in agreement with other studies that reported a loss of PTEN expression was linked to a decreased survival time in cases of uterine leiomyosarcomas [14].

In this study, NGS was used to determine the pattern of genomic instability in different pediatric sarcoma samples (OS, RMS, and ES). We detected abnormalities in the cell cycle and known mutations or gene fusions in the 54 pediatric sarcoma tissues analyzed. Amplifications of oncogenes were easily found; p53, PTEN, and PIK3CA were among the most prevalent detected mutations. It was postulated that human carcinogenesis had been linked to somatic mutations in PTEN. PTEN mutations were shown to be an effective indicator of cell proliferation in sarcomas in a prior investigation [16].

In this study, it was clearly noticed that although PTEN protein loss was detected in 54 cases, only 11 out of 54 cases had a deletion of PTEN at the genomic level. A recent study established that even in

the lack of PTEN gene mutations, decreased IHC expression of PTEN is commonly detected in cancer patients [17]. Another study described that PTEN somatic mutations were present in 4.8% of cases, while loss of PTEN protein expression was shown in 48% of breast cancer cases [7]. Based on our findings that came in accordance with these previously reported studies, PTEN loss might be caused by other processes such as methylation, loss of heterozygosity, and transcriptional or post-transcriptional profiling. These epigenetic changes of DNA were thought to play a critical function in the growth of soft tissue sarcomas as PTEN mutations were less common in soft tissue sarcomas than in other cancers [18, 19].

Cell proliferation and survival could be boosted by activating the phosphatidylinositol 30-kinase (PI3K)/AKT pathway. In view of the fact that PIK3CA mutations and loss of PTEN function were thought to activate the PI3K/AKT pathway, it is hypothesized that PIK3CA mutations and loss of PTEN expression are mutually exclusive in their effects [20]. Our results showed that mutations of the PIK3CA gene coexist with alterations of the PTEN gene in three out of 54 studied cases, suggesting that alterations in multiple genes may activate the PI3K/AKT pathway. PTEN deficiency causes a strong initiation of the AKT/mTOR pathways, which could contribute to the treatment strategy of those sarcoma cases, as numerous authorized inhibitors can be used as tailored treatments for individuals with PTEN-deficient malignancies [21-23].

Furthermore, p53 interaction with PI3K/AKT pathways plays a significant role in cell death/survival, PTEN plays a vital role in the regulation of p53-mediated apoptosis through its transcriptional regulation by P53 [24]. It is still unclear whether PIK3CA mutations and p53 mutations are associated, but it was mutually exclusive to have PIK3CA and P53 mutations [25]. There were six cases out of 54 in which both PIK3CA mutations and p53 mutations were found as double mutations

in our study, but this coexistence did not reach a significant association level. This finding may refer to a limited number of the studied cases. Nonetheless, this finding could indicate that the two pathways could be functionally redundant and complementary.

Regarding p53 association with PTEN gene loss in our studied sarcoma cases, seven out of 54 sarcoma tumor samples showed coexcistance of both PTEN and p53 mutations. several studies reported association interaction between P53 mutations and PTEN loss in sarcoma [18-22]. PTEN is a more recurrently mutant tumor suppressor gene with frequently repressed or downregulated expression. PTEN activity is frequently disturbed in cancer cells due to its pivotal role and pleiotropic nature [8]. Inactivation of PTEN causes cancerous tumors to lose function since it disrupts PI3K pathway activity, in addition to its catalytic phosphatase activity. Defects in PTEN can alter processes in the cell that are crucial for cancer development, such as survival, proliferation, energy metabolism, and cellular construction [26]. In terms of the association between different detected mutations and overall survival, neither a single gene mutation nor a combination of multiple mutations was associated with poor survival.

In the light of previous findings , our results cant highlight the possibility of coexistence of PTEN and other gene abnormalities. Additional microdeletions or rearrangements of other genes discovered in the current investigation cannot be ruled out. Other PTEN inactivation pathways, on the contrary, might play a significant role.

CONCLUSION

To our knowledge, this is the first study using NGS to assess genomic instability, highlighting the role of PTEN gene mutation in pediatric sarcoma cases and its association with loss of PTEN protein expression. In this investigation, our findings clearly suggest that PTEN downregulation plays a crucial role in the course of pediatric sarcoma. PTEN mutations are less common in soft tissue sarcomas than in other cancers, and this is clearly demonstrated in our findings that showed the presence of lack of correlation between increased PTEN protein expression and PTEN gene activating mutations results. These findings may be linked with the frequency of other actionable coexisting mutations, including p53 and PIK3CA. Predicting which patients have high levels of these molecular genetic changes means patients at high risk of sarcoma should be prioritized. Furthermore, research is urgently needed to elucidate the molecular mechanisms implicated in these cases and to which novel therapies may be directed. In order to achieve these research objectives, it is recommended that NGS-based genomic evaluation be added to the standard diagnostic.

Acknowledgments: The authors would like to thank the *Deanship of Scientific Research at Umm Al-Qura University for its continuous support.* This work was supported financially by *the Deanship of Scientific Research at Umm Al-Qura University to Dr.: Hassan Fouad Huwait [*Grant Number: MED-1-01-0002-17].

Data availability: Immunohistochemistry and NGS Data used to support the findings of this study are available from the corresponding author upon request.

Funding statement: The authors disclosed receipt of the following financial support for the research and authorship of this article: This work was supported by the Deanship of Scientific Research at Umm Al-Qura University (Grant Number: MED-1-01-0002-17).

Conflicts of interest: The Authors declare that there is no conflict of interest.

Authors' Contribution: Hassan Fouad Huwait and Hanan Mohammed Abd Elmoneim conceived and designed the study, provided research materials, and collected and organized data. Hanan Mohammed Abd Elmoneim, Mariana Fathy Gayyed, and Rabab Ahmed Moussa conducted the data analysis, interpreted it, and wrote the paper. Hanan Mohammed Abd Elmoneim and Rabab Ahmed Moussa wrote the initial draft and provided logistic SUPPORT. Hanan Mohammed Abd Elmoneim and Rabab Ahmed Moussa revises and finalizes the paper. All authors contributed equally to have critically reviewed and approved the final draft. All authors are responsible for the content and similarity index of the manuscript.

RFERENCES

1. Aljuhani WS, Alanazi AM, Alghafees MA. Primary bone sarcomas in KSA: A Saudi tumor registry review. Journal of Taibah University Medical Sciences 2021; 16: 77-85.
2. Burningham Z, Hashibe M, Spector L, Schiffman JD. The Epidemiology of Sarcoma. Clinical Sarcoma Research 2012; 2: 1-16.
3. Skapek SX, Ferrari A, Gupta AA et al. Rhabdomyosarcoma. Nature Reviews Disease Primers 2019; 5: 1-19.
4. Montella L, Altucci L, Sarno F et al. Toward a personalized therapy in soft-tissue sarcomas: State of the art and future directions. Cancers 2021; 13: 2359-2359.
5. Stefano S, Giovanni S. The PTEN tumor suppressor gene in soft tissue sarcoma. Cancers 2019; 11: 1169-1169.

6. Cuppens T, Annibali D, Coosemans A et al. Potential Targets' Analysis Reveals Dual PI3K/mTOR Pathway Inhibition as a Promising Therapeutic Strategy for Uterine Leiomyosarcomas - An ENITEC Group Initiative. Clinical Cancer Research 2017; 23: 1274-1285.

7. Li G, Guo X, Chen M et al. Prevalence and spectrum of AKT1, PIK3CA, PTEN and TP53 somatic mutations in Chinese breast cancer patients. PLoS ONE 2018; 13: e0203495-e0203495.

8. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nature Reviews Molecular Cell Biology 2012; 13: 283-296.

9. Choi JH, Ro JY. The 2020 WHO Classification of Tumors of Soft Tissue: Selected Changes and New Entities. Advances in Anatomic Pathology 2021; 28: 44-58.

10. Quattrone A, Wozniak A, Dewaele B et al. Frequent mono-allelic loss associated with deficient PTEN expression in imatinib-resistant gastrointestinal stromal tumors. Modern Pathology 2014; 27: 1510-1520.

11. Cote GM, He J, Choy E. Next-Generation Sequencing for Patients with Sarcoma: A Single Center Experience. The Oncologist 2018; 23: 234-242.

12. Gutiérrez-Jimeno M, Alba-Pavón P, Astigarraga I et al. Clinical value of NGS genomic studies for clinical management of pediatric and young adult bone sarcomas. Cancers 2021; 13: 5436-5436.

13. Cheng L, Pandya PH, Liu E et al. Integration of genomic copy number variations and chemotherapy-response biomarkers in pediatric sarcoma. BMC Medical Genomics 2019; 12: 89-106.

14. Movva S, Wen W, Chen W et al. Multi-platform profiling of over 2000 sarcomas: Identification of biomarkers and novel therapeutic targets. Oncotarget 2015; 6: 12234-12247.

15. Ricci R, Maggiano N, Castri F et al. Role of PTEN in Gastrointestinal Stromal Tumor Progression. Archives of Pathology and Laboratory Medicine 2004; 128: 421-425.

16. Kim J, Kim JH, Kang HG et al. Integrated molecular characterization of adult soft tissue sarcoma for therapeutic targets. BMC Medical Genetics 2018; 19: 1-11.

17. Pulido R, Mingo J, Gaafar A et al. Precise immunodetection of PTEN protein in human neoplasia. Cold Spring Harbor Perspectives in Medicine 2019; 9: a036293-a036293.

18. Yin L, Cai WJ, Liu CX et al. Analysis of PTEN Methylation Patterns in Soft Tissue Sarcomas by MassARRAY Spectrometry. PLoS ONE 2013; 8: e62971-e62971.

19. Yin L, Liu CX, Nong WX et al. Mutational analysis of p53 and PTEN in soft tissue sarcoma. Molecular Medicine Reports 2012; 5: 457-461.

20. Saal LH, Holm K, Maurer M et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Research 2005; 65: 2554-2559.

21. Castillo-Martin M, Thin TH, Lorduy AC, Cordon-Cardo C. Immunopathologic assessment of PTEN expression. Methods in Molecular Biology 2016; 1388: 23-37.

22. Jung SH, Hwang HJ, Kang D et al. mTOR kinase leads to PTEN-loss-induced cellular senescence by phosphorylating p53. Oncogene 2019; 38: 1639-1650.

23. Papa A, Pandolfi PP. The pten–pi3k axis in cancer. Biomolecules 2019; 9: 153-153.

24. Maruyama N, Miyoshi Y, Taguchi T et al. Clinicopathologic analysis of breast cancers with PIK3CA mutations in Japanese women. Clinical Cancer Research 2007; 13: 408-414.

25. Singh B, Reddy PG, Goberdhan A et al. p53 regulates cell survival by inhibiting PIK3CA in squamous cell carcinomas. Genes and Development 2002; 16: 984-993.

26. Vidotto T, Melo CM, Castelli E et al. Emerging role of PTEN loss in evasion of the immune response to tumours. British Journal of Cancer 2020; 122: 1732-1743.