Role of expression of P53, Cyclin D1 and Epidermal growth factor receptor (EGFR) in some benign, intermediate and malignant spindle cell soft tissue tumors

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Abstract: It has been found that expression of P53, cyclin D1 and Epidermal growth factor receptor (EGFR) in spindle cell soft tissue tumors (SCSTTs) are correlated with histological grade and assessment of tumor aggressiveness. The aim of this study was to evaluate the immunohistochemical expression of p53, cyclin D1 and Epidermal Growth Factor Receptors (EGFR) in some spindle cell soft tissue tumors and their association with histological grade of these tumors in an attempt to predict the biologic behavior of these tumors.This work included 33cases previously diagnosed spindle cell soft tissue tumors (16 males (48.48%) and 17 females (51.52 %) and 3 control cases. Patients' age ranged from 20 to 60 years. This work included 3 groups of SCSTTs; 1st. group (4 cases) (12.12%) with benign tumors; one case of neurofibroma (3.03), one case of deep benign fibrous histiocytoma (3.03), one case of angiomatoid fibrous histiocytoma (3.03), and one case of Schwannoma (3.03). 2nd. Group (6 cases) (18.18%) with intermediate grade malignant SCSTTs among them 4 cases of dermato-fibrosarcoma protuberans (12.2%) and 2 cases of desmoid fibromatosis (6.06%).3rd. group: (23 cases) (69.6%), with malignant SCSTTs this group composed of 9 cases of synovial sarcoma (27.27%), 4 cases of leiomyosarcoma (12.1%), 3 cases of malignant peripheral nerve sheath tumor (9.09%), and 7 cases of malignant fibrous histiocytoma (21.2%). Four sections were prepared from each specimen and stained with hematoxyllin & eosin, for routine microscopic examination, as well as, immunostaining for P53, Cyclin D1 and Epidermal growth factor receptor (EGFR). immunoreactivity were calculated. A significant difference in immunoreactivity among various histologic patterns of benign, intermediate grade malignant and malignant SCSTTs was detected. Statistical analysis showed that this increase is not significant except in comparing the behavior of soft tissue tumors. This study indicated that the degree of positivity to P53, Cyclin D1 and Epidermal growth factor receptor (EGFR) may play a great role in detection of behavior and prognosis of different types of spindle cell soft tissue tumors and can differentiate between benign, intermediate grade malignant and malignant spindle soft tissue tumors. However, further studies on larger scales are recommended.

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Key words: P53, Cyclin D1 , Epidermal growth factor receptor (EGFR), spindle cell soft tissue tumors

1.Introduction:

Soft tissues can be defined as non-epithelial extra skeletal tissues of the body exclusive of the reticuloendothelial system, glia and supporting tissue of various parenchymal organs (Weiss and Goldblum, 2001). Soft tissue tumors constitute a large and heterogenous group of neoplasms (Rosai, 2004) The large majority of soft tissue tumors are benign, with a very high cure rate after surgical excision. Malignant mesenchymal neoplasms amount to less than 1% of the overall human burden of malignant tumors but they are life-threatening and often associated with unique clinical, prognostic and therapeutic features (Rosenberg, 2005). Cytological classification of soft tissue tumors based on the principal pattern includes five groups; pleomorphic pattern, spindle cell pattern, myxoid pattern, small round/ovoid cell pattern and epithelioid pattern. Spindle cell soft tissue tumors (SCSTTs) are special types of soft tissue tumors that are characterized by spindle cells which are shed as fascicles. A typical spindle cell has fusiform or ovoid nuclei; the cytoplasm is tapered, unipolar, or bipolar; mitotic figures are variable (Akerman and Domanski, 2003). Complete surgical excision is the only curative treatment for the vast majority cases of SCSTTs. The overall 5-year survival rate of SCSTTs is about 60%, but biological behavior of SCSTTs is highly variable. Certain histological types of sarcoma have an indolent clinical course with low metastatic potential.Whereas, other types characteristically pursue a very aggressive course (Vesely et al., 2009). The key element of survival is control of both local recurrence ,as well as, distant metastasis. The histopathological grade is an important prognostic factor, 10-year disease specific survival for high-grade SCSTTs is 55% compared with 90% for low-grade tumors (Borden et al., 2003). New therapeutic strategies of cancer treatment directed against growth factor receptors such as the epidermal growth factor receptor (EGFR) are currently in use in the management of various carcinomas. Experimental

results have given clues that blockage of EGFR mediated pathways might also be of therapeutically benefit in soft tissue sarcomas *(Ranson, 2004)*

The aim of this study was to evaluate the immunohistochemical expression of p53, cyclin D1 and Epidermal Growth Factor Receptors (EGFR) in some spindle cell soft tissue tumors and their association with histological grade of these tumors in an attempt to predict the biologic behavior of these tumors.

2.Materials and Methods:

2.1.Materials:

2.1.1.Subjects:

This study involved 36 cases; 3 control cases (1 case of well differentiated squamous cell carcinoma of skin for EGFR; 1 case of infiltrating duct carcinoma of breast for cyclin D1; 1 case of colonic adenocarcinoma for p53) and 33 cases of previously diagnosed SCSTT retrieved from the surgical files of the Histopathology Department, Al-Azhar University hospitals.

The clinical data of the patients were obtained from the pathology reports and from patient's charts.

2.1.2. Samples :

From each paraffin block, four sections (5 micron each) were prepared for routine hematoxyllin and eosin (H&E) staining, and immunostaining for P53, Cyclin D1 and Epidermal growth factor receptor (EGFR). Sections stained by H&E were examined and re-evaluated and the cases were classified according to WHO histologic classification of soft tissue tumors (2002), and graded according to FNCLCC grading system (Fletcher et al., 2002).

2.2.Methods:

2.2.1.Microscopic examination of tumor cells:

Sections which were immunostained for p53 (Thermo Scientific-LabVision-USA-clone-DE-M-16-colorectal cancer-ready to use), cyclin D1 (Thermo Scientific -LabVision-USA-clone DE-R-11 breast carcinoma- ready to use) and epidermal growth factor receptor (Thermo Scientific -LabVision-USA-clone R4A-squamous cell carcinoma -ready to use) were examined microscopically to detect positively-stained tumor cells. Positive p53 and cyclin D1 staining was nuclear while positive epidermal growth factor receptor staining was cytoplasmic or membranous and both appeared brown in color.

2.2.2. Semi-quantitative scoring for stained tumor cells :

A semiquantitative scoring system was performed according to the percentage of stained tumor cells :

a- *For p53 : negative (no stains)* ;

*+1 (weak) staining of < 33% of tumor cell nuclei ,

*+2 (moderate) staining of 34-67% of tumor cell nuclei and

*+3(strong) staining of > 67% of tumor cell nuclei **(** Gary et al., 2002).

***b-For Cyclin D1: negative (no stains)* ,**

*+1 (weak) staining of < 10% of tumor cell nuclei ,.

*+2(moderate) staining of 10-50% of tumor cell nuclei and

*+3(strong) staining of > 50% of tumor cell nuclei (Nurija et al., 2005).

***c-For EGFR:* negative (no stains),** :

*+1 (weak) for faint partial membrane staining,

*2+ (moderate) for weak complete membrane staining in >10% of cells,

*3+ (strong) for intense complete membrane staining in >10% of cells **(**Vesely et al., 2009).

2.2.3.Statistical analysis :

Statistical analysis of the data was performed by the statistical software. The relation between marker expression and histological grade was studied by applying $\chi 2$ test, Fisher exact test, Spearman correlation test and Mann-Whitney test and $P < 0.05$ was defined as the level of significance.

3.Results:

3.1.Histopathology:

3.1.1.-Benign tumors: (4 cases)

Neurofibroma (1 case), angiomatoid fibrous histiocytoma (1 case), deep benign fibrous histiocytoma (1case), and schwannoma (1 case)

3.1.2.-Tumors of intermediate grade of malignancy: (6 cases)

Dermatofibrosarcoma protuberans (4 cases) and desmoid-type fibromatosis (2 cases)

3.1.3.-Malignant tumors: (23 cases)

a--Synovial sarcoma: (9 cases)

Biphasic synovial sarcoma (1 case) and monophasic synovial sarcoma (8 cases)

b--Leiomyosarcoma: (4 cases)

c-Malignant peripheral nerve sheath tumor:
(3 cases)

d-Malignant fibrous Histiocytoma (MFH):
(7 cases)

Pleomorphic MFH (5 cases) and Myxoid MFH
(2 cases)

3.2.- Immunohistochemical findings:

3.2.1. P53 expression :

Immunoreactivity was detected as brownish discoloration of the nuclei of the tumor cells.

P53 was expressed in 10 out of 33 cases of spindle cell soft tissue tumors examined (30.30%).

-Benign tumors (4 cases)

P53 was expressed in 1 out of 4 benign tumors(25%) and the expression was moderate (2+), while the other three cases showed negative p53 expression.

-Tumors of intermediate grade of malignancy (6 cases)

P53 was expressed in 1 out of 6 intermediate grade of malignant tumors (case of desmoid fibromatosis)

(16.66%) and the expression was moderate (2+)while the other cases showed negative P53 expression.

-Malignant tumors (23 cases)

P53 was expressed in 8 out of 23 cases of malignant SCSTT (34.78%) which included 2 out of 9 cases of synovial sarcoma (22.22%), 2 out of 4 cases of leiomyosarcoma (50%), and 4 out of 7 cases of malignant fibrous histiocytoma (57.14%). The expression ranged from weak (1+) in one case to moderate (2+) in three cases and strong (3+) in four cases. P53 was not expressed in any of the malignant peripheral nerve sheath tumors examined. Table (10).

Table (1): P53 expression in malignant SCSTT: (23 cases)

Group			P53					No. of positive cases to:	
			Negative	Weak	Moderate	Strong	Total	23 malignant cases	33 studied cases
Malignant SCSTT	Synovial sarcoma	No	7	0	1	1	9	2\23	2\33
		%	77.77	0.00	11.11	11.11	100%	8.69%	6.06%
	Leiomyosarcoma	No	2	0	1	1	4	2\23	2\33
		%	50%	0.00	25%	25%	100%	8.69%	6.06%
	MPNST	No	3	0	0	0	3	0\23	0\33
		%	100%	0.00	0.00	0.00	100%	0.00	0.00
	Malignant fibrous histiocytoma	No	3	1	1	2	7	4\23	4\33
		%	42.85	14.28	14.28	28.57	100%	17.39	12.12
Total		No	15	1	3	4	23	-	-
		%	45.45	3.03	9.09	12.12	69.69	-	-

When correlation was done between P53 expression and tumor grade, it was expressed in 3 out of 11 cases of grade II tumors (27.27%) and was expressed in 5 out of 12 cases (41.66%) of grade III tumors. A statistically significant relationship was formed between P53 expression and tumor grade with p value = 0.040 < 0.05, Table(2)

Table (2): P53 expression in relation to grade of malignant SCSTT:

Group			GII	GIII	Total
No.			11	12	23
Malignant SCSTT	Synovial sarcoma	No.	0	2	2
		%	0.00	16.66	8.69
	Leiomyosarcoma	No.	1	1	2
		%	9.09	8.33	8.69
	MPNST	No.	0	0	0
		%	0.00	0.00	0.00
	Malignant fibrous Histiocytoma	No.	2	2	4
		%	18.18	16.66	17.39
Total		No.	3	5	8
		%	27.27	41.45	34.78
Chi-square		X^2	15.156		
		P-value	0.040*		

3.2.2.Cyclin D1 expression:

Immunoreactivity was detected as brownish discoloration of the nuclei of the tumor cells.

The expression of cyclin D1 was found in 11 out of 33 cases of soft tissue spindle cell tumors (33.33%).

-Benign tumors (4 cases)

Cyclin D1 was not expressed in any of the tumors.

-Tumors of intermediate grade of malignancy (6 cases)

Cyclin D1 was not expressed in any of the intermediate grade SCSTT.

-Malignant tumors (23 cases)

Cyclin D1 was expressed in 11 out of 23 cases of malignant SCSTT s (47.82%) which included 5 out of 9 cases of synovial sarcoma (55.55%), 3 out of 4 cases of leiomyosarcoma (75%) and 3 out of 7 cases of malignant fibrous histiocytoma (42.85%). The expression ranged from weak (+1) in three cases, moderate (2+) in four cases and strong (3+) in four cases. Cyclin D1 was not expressed in any of the malignant peripheral nerve sheath tumor, Table (3)

Table (3): Cyclin D1 expression in malignant SCSTT: (23 cases)

Group			Cyclin D1					No. of positive cases to:	
			Negative	Weak	Moderate	Strong	Total	23 malignant cases	33 studied cases
Malignant SCSTT	Synovial sarcoma	No.	4	3	0	2	9	5\23	5\33
		%	44.44	33.33	0.00	22.22	100	21.73%	15.15
	Leiomyosarcoma	No.	1	0	2	1	4	3\23	3\33
		%	25	0.00	50	25	100	13.04	9.09
	MPNST	No.	3	0	0	0	3	0\23	0\33
		%	100	0.00	0.00	0.00	100	0.00	0.00
	Malignant fibrous histiocytoma	No.	4	0	2	1	7	3\23	3\33
		%	57.14	0.00	28.57	14.28	100	13.04	9.09
Total		No.	12	3	4	4	23	-	-
		%	36.36	9.09	12.12	12.12	69.69	-	-

When correlation was done between cyclin D1 expression and tumor grade, it was expressed in 4 out of 11 cases of grade II tumors (36.36%) and in 7 out of 12 cases of grade III tumors (58.33%). A statistically significant relationship was found between cyclin D1 expression and tumor grade with p value = 0.014< 0.05, table(4).

Table (4): Cyclin D1 expression in relation to grade of malignant SCSTT

Group			GII	GIII	Total
No.			11	12	23
Malignant SCSTT	Synovial sarcoma	No.	2	3	5
		%	18.18	25.00	21.73
	Leiomyosarcoma	No.	1	2	3
		%	9.09	16.66	13.04
	MPNST	No.	0	0	0
		%	0.00	0.00	0.00
	Malignant fibrous histiocytoma	No.	1	2	3
		%	9.09	16.66	13.04
Total		No.	4	7	11
		%	36.36	58.33	47.82
Chi-square		X^2	23.798		
		P-value	0.014*		

3.2.3.EGFR expression:

Immunoreactivity (membranous and cytoplasmic)

EGFR was expressed in 7 out of the 33 cases of SCSTT examined (21.21%).

-Benign tumors (4 cases)

EGFR was not expressed in any of the benign tumors.

-Tumors of intermediate grade of malignancy (6 cases) EGFR was not expressed in any of these tumors.

-Malignant tumors (23 cases)

The expression of the EGFR was found in 6 out of 23 cases of malignant SCSTT s (26.08%) which included 2 out of 9 cases of synovial sarcoma (22.22%), 3 out of 4 cases of leiomyosarcoma (75%) and 1 out of 7 cases of malignant fibrous histiocytoma (14.28%). The expression ranged from moderate (2+) in three cases to strong (3+) in three cases. EGFR was not expressed in any of the cases of malignant peripheral nerve sheath tumors examined, Table(5).

Table (5): EGFR expression in malignant SCSTT: (23 cases)

Group			EGFR					No. of positive cases to:	
			Negative	Weak	Moderate	Strong	Total	23 malignant cases	33 studied cases
Malignant SCSTT	Synovial sarcoma	No.	7	0	1	1	9	2\23	2\33
		%	77.77	0.00	11.11	11.11	100	8.69%	6.06%
	Leiomyosarcoma	No.	1	0	2	1	4	3\23	3\33
		%	25	0.00	50	25	100%	13.04	9.09
	MPNST	No.	3	0	0	0	3	0\23	0\33
		%	100	0.00	0.00	0.00	100	0.00	0.00
	Malignant fibrous histiocytoma	No.	6	0	0	1	7	1\23	1\33
		%	85.71	0.00	0.00	14.28	100	4.34	3.03
Total		No.	17	0	3	3	23	-	-
		%	51.51	00.00	9.09	9.09	69.69	-	-

When correlation was done between EGFR expression and tumor grade, it was expressed in 1 out of 11 cases of grade II tumors (9.09%) and in 5 out of 12 cases of grade III tumors (41.66%) and the results were statistically significant with p value = 0. 025< 0.0 Table (6 & 7)

Table (6): EGFR expression in relation to grade of malignant SCSTT

Group			GII	GIII	Total
No.			11	12	23
Malignant SCSTT	Synovial sarcoma	No.	0	2	2
		%	0.00	16.66	8.69
	Leiomyosarcoma	No.	1	2	3
		%	9.09	16.66	13.04
	MPNST	No.	0	0	0
		%	0.00	0.00	0.00
	Malignant fibrous histiocytoma	No.	0	1	1
		%	00.00	8.33	8.33
Total		No.	1	5	6
		%	9.09	41.45	26.08
Chi-square		X^2	14.340		
		P-value	0.025*		

Table (7): Correlation between P53, Cyclin D1 and EGFR expression in malignant SCSTT:

Groups	No.	Type	P53				Cyclin D1				EGFR			
			-ve	1+	2+	3+	-ve	1+	2+	3+	-ve	1+	2+	3+
Malignant SCSTT	9	Synovial sarcoma	7	0	1	1	4	3	0	2	7	0	1	1
	4	Leiomyosarcoma	2	0	1	1	1	0	2	1	1	0	2	1
	3	MPNST	3	0	0	0	3	0	0	0	3	0	0	0
	7	Malignant fibrous histiocytoma	3	1	1	2	4	0	2	1	6	0	0	1
Total	23		22	1	3	4	19	3	4	4	24	0	3	3

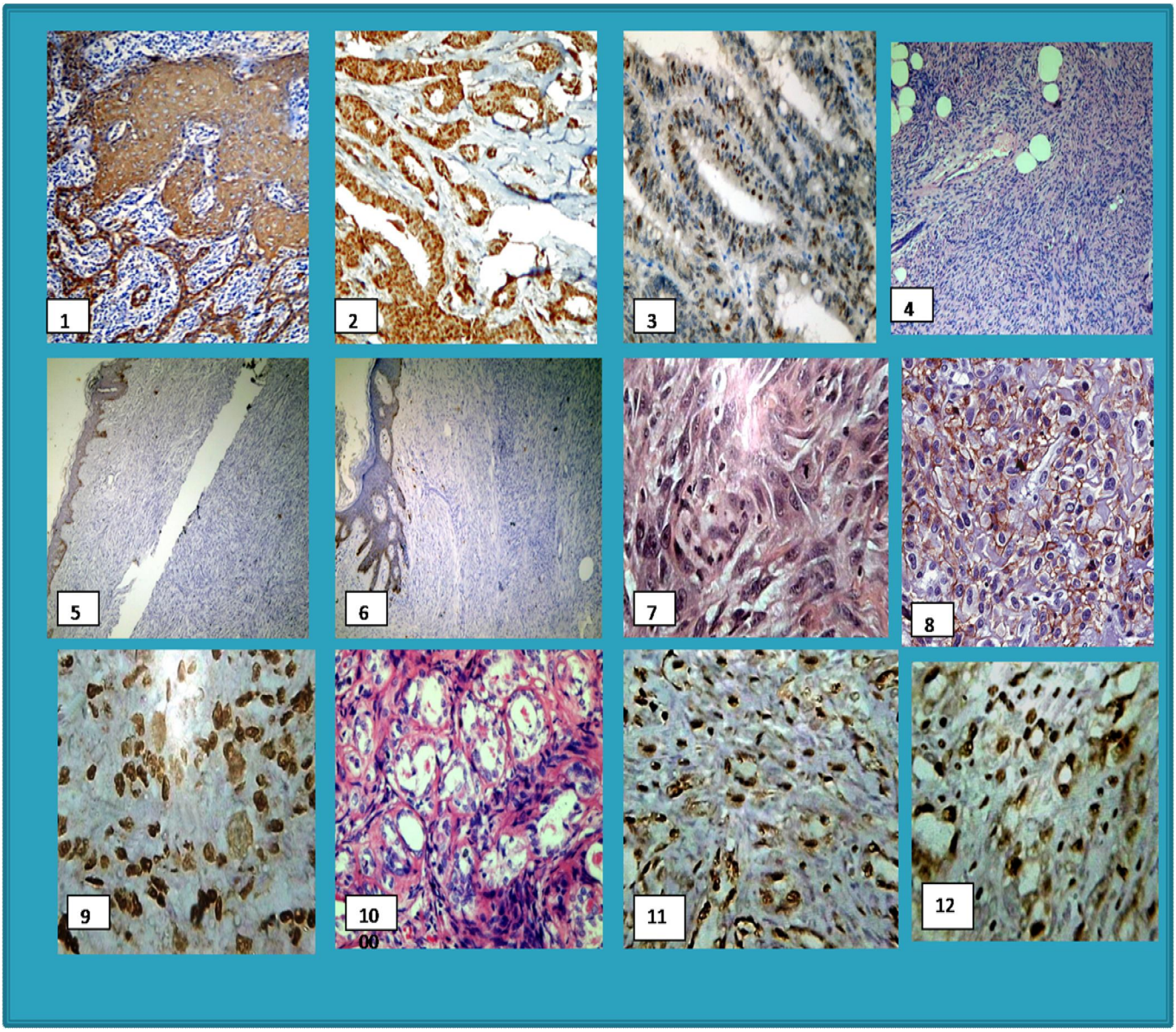

Fig.1: Well differentiated squamous cell carcinoma (control case) showing strong EGFR immunoreactivity (membranous and cytoplasmic) (ABC x100)

Fig.2: **Infiltrating duct carcinoma (control case) showing strong nuclear cyclin D1 immunoreactivity** (ABC x200)

Fig.3: **Colonic adenocarcinoma (control case) showing moderate p53 immunoreactivit(ABC-x 250)**

Fig.4: **Dermatofibrosarcoma protuberans showing interwoven boundless of spindle cells arranged in a storiform pattern infiltrating fat (H&E.x100)**

Fig5: **Dermatofibrosarcoma protuberance showing no P53 immunoreactivity (ABC x40)**

Fig 6: **Dermatofibrosarcoma protuberance showing no EGFR immunoreactivity (ABC x 40)**

Fig7: **Pleomophic MFH (H &E x250)**

FIG 8: **Pleomophic MFH showing strong EGFR immunoreactivity (3+) membranous staining (ABC x 250)**

FIG 9: **Pleomophic MFH showing strong cyclin D1 immunoreactivity (3+) nuclear staining (ABC x 360)**

Fig10: **Biphasic synovial sarcoma showing both epithelial and spindle cell components ((H&E.x200)**

Fig11: **Biphasic synovial sarcoma showing moderate P53 immunoreactivity (2+) nuclear staining in epithelial and spindle cell components (ABC x250)**

Fig12: **Biphasic synovial sarcoma showing moderate Cyclin D1 immunoreactivity (2+) nuclear staining in epithelial and spindle cell components (ABC x250)**

4-Discussion

In this study, 33 primary untreated SCSTTs were included. P53 was expressed in 25% of benign tumors as compared to 16.66% expression in intermediate tumors, and it was expressed more frequently in malignant tumors, where 27.27% were positive in grade II malignant SCSTT tumors and 41.45% in grade III malignant SCSTT tumors. The frequency of P53 positivity significantly increased with increase of histologic grade ($p = 0.040$). In this work a direct relationship was found between P53 intensity of expression and tumor grade but it was statistically insignificant with (p value=0.305 These findings are in accordance with those of Dei et al., (1993) who found that P53 was expressed in 48% of benign/reactive soft tissue lesions, 21% of intermediate malignant soft tissue tumors and 65% of malignant soft tissue tumors. They concluded that P53 immunoreactivity in sarcomas can be interpreted as a mutation of the corresponding P53 gene, suggesting that its alteration may have a role in their pathogenesis and although they did not exclude P53 in benign/reactive soft tissue lesions, yet it was difficult to sustain this hypothesis. Similar findings were reported by Jensen et al., (1998) who found P53 immunoreactivity in 24% and 26% of GI and GII soft tissue sarcomas Sabah et al., (2007) studied one hundred fifty-two cases of different types of soft tissue sarcomas. The cases consisted of 54 low-grade, 40 intermediate-grade, and 58 high-grade sarcomas. Nuclear reactivity for P53 was detected in 49 cases (32.2%). p53 expression correlated with the tumor grade (low grade, 5.6%; intermediate grade, 22.5% and high grade 63%). It was concluded that expression of p53 is closely associated with the histologic grade of the tumor, and therefore, these proteins may be used as prognostic markers.

In the current study, increased p53 expression with increased tumor grade supports the role of p53 mutation in tumor aggressiveness. Cyclin D1 was not expressed in all benign tumors and intermediate spindle cell soft tissue tumors. Expression of Cyclin D1was significantly more frequent in high-grade tumors, where 37% of grade II tumors were positive and 59% of grade III tumors exhibited cyclin D1 nuclear immunostaining. The frequency of Cyclin D1 positivity significantly increased with increase of histologic grade ($p = 0.014$). In this work a direct relationship was formed between cyclin D1 intensity of expression and tumor grade but it was statistically insignificant (p value = 0.305).

The results are also in concordance with those of Kim et al., (1998) who also observed increased expression of cyclin D1 in 29% of these cases and the expression was related to histological grade. The results are also consistent with the findings of Vesely et al., (2009) who studied 101 patients with adult STSs. The expression of the cyclin D1 was found in 29 cases (28.7%). None of the grade I sarcomas were positive for cyclin D1, whereas (32.1%) of the grade II and (34.5%) of grade III tumors showed positivity. The cyclin D1 expression showed a statistical association with tumor grade ($p = 0.028$).

In the current study, EGFR was expressed in 21% of SCSTTs. EGFR was not expressed in all benign tumors and intermediate spindle cell tumors. EGFR was expressed in 16% of grade II malignant tumors and in 41% of grade III malignant tumors and the result were statistically significant ($p = 0.025$). A direct relationship was formed between the intensity of EGFR expression and tumor grade but it was statistically insignificant ($p = 0.954$). Similar finding were reported by Sato et al., (2005) who found that EGFR was expressed in about 45% of spindle cell soft tissue tumors that examined and they demonstrated a significant correlation between expression and tumor grade. Results agree with the findings of Yang et al., (2006) who studied 46 of STS and EGFR was positively expressed in 78% of STS samples that consisted of different histological types. In the current study, a statistically significant relationship was formed between the EGFR and tumor grade.

Conclusion:

In this study, it has been found that expression of P53, cyclin D1 and EGFR in SCSTTs correlated with histological grade, indicating their valuable contributing to the assessment of tumor aggressiveness and to the establishing of histological grade in soft tissue sarcoma patients, especially in small tissue samples. We also recommend that other studies with large number of cases may be more useful and reliable.

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