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Role of P16, P53 and Cyclin D1 in Detection of HPV Induced Laryngeal Squamous Cell Carcinoma: An Immunohistochemical Study

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ABSTRACT

Background: Laryngeal squamous cell carcinoma is one of the most relevant neck malignancies that critically endanger health and even the life of humans. There is a strong need to identify specific markers of Laryngeal squamous cell carcinoma that are supposed probably to have valuable prognostic value, furthermore, even may show a great aid in designing the treatment plan.
Aim of the study: Our study aimed to study the expression of p16, in Laryngeal squamous cell carcinoma and correlate it with different clinical and pathological parameters and to study the expression of other immune-histochemical markers such as P35 and Cyclin d1 about the expression of p16, in squamous cell carcinoma of the larynx.
Patients and Methods: This retrospective study included 50 Paraffin blocks that were obtained from previously diagnosed squamous cell laryngeal carcinoma patients during the period of the research from the Pathology Department, Faculty of Medicine, Tanta University.
Results: The expressions of P16, P53, and Cyclin d1 in the squamous cell laryngeal carcinoma samples were detected. It has been found that 90% of samples were positive for P16, 78% were positive for P35 and 50% were positive for Cyclin d1. P16 expression developed a statistically significant correlation with the tumor extension and certain histopathological types, (P=0.024) and (P=0.007), respectively.
Conclusion: P16**,** P35, and Cyclin d1 are considered to be indirect markers for Human papillomavirus (HPV) related Laryngeal squamous cell carcinoma.

Keywords: *P16, P35, and Cyclin D1; Human papilloma virus; Laryngeal Squamous Cell Carcinoma.*

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INTRODUCTION

Laryngeal carcinoma prevalence is increasing over time worldwide. In the past few decades, the incidence of laryngeal carcinoma has been rising steadily in the USA. The Middle East Cancer Consortium stated that Egypt has one of the most frequently documented cases of cancer larynx among the Middle East Cancer Consortium countries.[1]

Head and Neck Squamous Cell Carcinomas, include cancers of the aero-digestive tract, an estimated annual burden of thousands of deaths, and more than 650,000 incident cases, making it the sixth most common cancer worldwide, studies have shown that Head & Neck Squamous cell carcinomas (HNSCCs) responsible for about 20% of all cancers. [2, 3]

In Egypt, the Cancer Pathology Registry of the National Cancer Institute of Cairo University declared that during the years 2003/2004 laryngeal cancer was the most frequent among all respiratory cancers (27.84%) and the tenth most frequent among all cancers (1.77%), with most cases diagnosed at advanced stages. [4, 5]

Laryngeal cancer can result from the combined action of smoking, drinking alcohol, air pollution, and gastroesophageal reflux. Within the Middle East, smoking rates are high, although alcohol consumption is ruled harsh, this is especially true for Egypt, where smoking rates are increasing for both cigarettes and water pipes. [6]

Infection with various Human papilloma virus types is related to the development of laryngeal carcinoma independent of tobacco and alcohol use. Human papilloma virus infection plays a role in the molecular pathways through its viral oncoproteins; E6 and E7, these two proteins increase the degradation of p53 and interfere with Retinoblastoma

protein (pRb) function, causing up-regulation of p16 by losing negative-feedback control, a mutation in the p16-encoding gene leads to p16 overexpression, which has been demonstrated in many cervical and head and neck cancers.[7]

The effect of Human papilloma virus on laryngeal carcinoma prognosis and whether HPV-associated laryngeal carcinoma carries a better or worse outcome; P16 immuno-expression, a surrogate marker for Human papillomavirus infection, often parallels the Human papilloma infection status in laryngeal squamous cell carcinoma, thus, by comparing P16 immunoreactivity with different prognostic markers of laryngeal carcinoma, the prognostic effect of Human papilloma virus on laryngeal carcinoma can be predicted. [8]

Immunohistochemical expressions of cyclin d1 and p53 were studied as prognostic markers of tumor proliferation in laryngeal carcinoma. P16 immunohistochemical staining study was performed as a marker for Human papilloma virus infection and compared with different prognostic markers such as patient's age, tumor grade, lymph node metastasis, lymph vascular invasion, thyroid cartilage infiltration and immunohistochemical markers of tumor proliferation such as cyclin D1 and p53.[9]

Our study aimed to study the expression of p16 in Laryngeal squamous cell carcinoma and correlate it with different clinical and pathological parameters and to study the expression of other immune-histochemical markers such as. P53 and Cyclin d1 concerning the expression of P16 in squamous cell carcinoma of the larynx.

PATIENTS AND METHODS

After approval of the local research ethics committee in both Al- Azhar and Tanta faculties of medicine, this study comprised a total of 50 formalin-fixed paraffin-embedded blocks of cases that had been diagnosed as laryngeal squamous cell carcinoma according to the latest 2017 WHO[10] classification system, subclassified into; conventional, basaloid and papillary squamous cell carcinoma, gathered retrospectively from the files in the medical archive of the department of pathology, Faculty of Medicine, Tanta University, from January 2020 till January 2021, all the tumor specimens were obtained during surgery and one representative paraffin block was selected for each patient.

After a meticulous revising of the medical archived records as clinical admission sheets & pathology diagnosis reports, the following data were collected:

Personal and present histories were acquired, as well as names, ages, genders, addresses, medically significant behaviors (smoking, alcohol abuse), and symptoms of interest such as voice and airway disturbances., General medical, oncological, and medical condition history.

Clinical examination of patients on admission included the search for any neck swellings.

A preoperative flexible fiberoptic laryngoscope report describes the site, the extension of the lesion, adequacy of the airway, and the mobility of the vocal folds that directly affect the staging of the tumor.

All patients had preoperative computed tomography scans to evaluate the tumor's actual size, extension, and cartilage invasion if found, as well as to detect any extra laryngeal dissemination (according to the tumor-node-metastasis (TNM) classification).

The paraffin wax blocks containing the selected specimens has been cut of 5µ and subjected to ordinary hematoxylin and eosin. staining for meticulous examination to confirm the histopathological diagnosis and to evaluate various histological features including.

a) Laryngeal carcinoma specimens were classified microscopically according to the (WHO 2017)[10] classification system

b) According to differentiation, studied laryngeal carcinoma specimens got graded by the traditional system according to the recommendations of the Union for international cancer control (UICC). The histological grades of tumors samples have gotten subdivided to Grade 1: Well, differentiated, Grade 2: Moderately differentiated, and Grade 3: poorly differentiated.[9]

c) Staging of studied laryngeal carcinomas were decided according to the recommendations of the American Joint Committee of Cancer (AJCC) using TNM staging; detection of other histological features as the depth of invasion (T), lymph node status (N), vascular and perineural invasion. [11]

Immunohistochemical methods:

An immunohistochemical study performed about laryngeal squamous cell carcinoma specimens and control specimens to detect the immunohistochemical expression of p16, cyclin d1, and p53 in the studied cases as follows: (1) Immunohistochemical stain for detection of p16 marker, (2) Immunohistochemical stain for detection of cyclin d1 marker, and (3) Immunohistochemical stain for detection of p53 marker:

The immunostaining procedure had done as follows: deparaffinization and rehydration of sections, blocking endogenous peroxidase, antigen retrieval, blocking non-specific staining, exposure to the primary antibody, exposure to secondary biotinylated antibody, exposure to streptavidin enzyme label, preparation of the working color reagent, and color development.

Immunohistochemical evaluation

1) P16

Cervical squamous cell carcinoma samples were used as the positive control, only cells showing nuclear staining were considered positive. P16 staining intensity was scored as follows: score1 (staining is less than 10% of tumor cells), score2 (staining in 10-50% of tumor cells) & score 3 (staining in more than 50% of tumor cells)[12].

2) Cyclin d1

Mantle cell lymphoma samples were used as the positive control, positive staining was considered only in the nuclei of tumor cells, the distribution of positive cells in tumors was also scored as follows: score 1 (staining in less than 10% of tumor cells), score 2 (staining in 10–50% of tumor cells) and score 3 (staining in more than 50% of tumor cells). Finally, cases were divided into positive and negative, cyclin d1 positive were cases showing moderate or intense nuclear staining in greater than or equal to 10% of tumor cells[13].

3) P53

Colonic carcinoma samples were used as the positive control, when greater than 10% of nuclear-stained tumor cells were detected in the section, the case was considered to be p53 positive[14].

Statistical analysis:

The previously described clinical, histological, and immunohistochemical data were all gathered and entered the 20th edition of the Statistical Package for Social Science. For quantitative data with a parametric distribution, mean, standard deviations, and ranges, and median with (IQR) for quantitative data with a non-parametric distribution. As generally, the Chi-square test was used, but when the predicted count in any cell was found to be less than 5, the Fisher exact test was used instead. The Mann-Whitney t-test and the independent t-test were used to compare two groups with quantitative data and parametric distribution. Ninety-five percent was set to be as the confidence interval and a very narrow margin of error accepted set to be about 5%, a statistically significant correlation is defined as a P-value of less than 0.05.[15]

RESULTS

This was a retrospective cohort study included 50 formalin-fixed paraffin-embedded blocks of cases that had been diagnosed as laryngeal squamous cell carcinoma cases (LSCC), showing immunohistochemical demonstration of Human papilloma virus using histochemical markers, our study correlations were set according to the following data:

Demographic data & risk factors:

The mean age items was 58.9 ± 11.18 years, with a range of 31 to 82 years, most of the patients were in the 40-70 years old interval, with a median of 56 years. The studied cases showed male to female predominance by 9:1 ratio (45 males:5 females).

40 cases (80%) gave history of smoking while, 10 cases (20%) gave no history of such habit.

cases (8%) gave history of drinking alcoholics on regular basis while, 46 cases (92%) showed complete abstinence. 6 cases (12%) showed related family history while, in the rest of the 44 cases (88%) there was no relevant family history.

Macroscopic data& histopathological types:

Tumor location: supraglottic 34% (17 cases), glottic 44% (22 cases) & subglottic in 22% (11 cases).

Tumor extension (macroscopic involvement of other tissues): thyroid cartilage involvement was found in only 24 (48%) cases out of the 50 studied cases (specimens) harvested intentionally with underlying cartilage.

Gross appearance: most of the studied cases appeared grossly as either of the following forms n (%); ulcerating masses 18 cases (36%), endophytic infiltrating annular masses 16 cases (34%), exophytic fungating or polypoidal masses 16 cases (32%).

Histopathological types: studied laryngeal squamous cell carcinoma cases were classified into cases of conventional type (21) cases, (11) cases were papillary type & (18) cases were of basaloid type.

Grading & staging

Tumor grading: among the studied laryngeal carcinoma cases & according to the grading system, we found that grade 1 (well differentiated) represented by 11 cases (22%), grade 2 (moderately differentiated) represented by 24 cases (48%), grade 3 (poorly differentiated represented by 15 cases (30%).

Tumor staging (TNM): according to the latest staging guidelines recommended by the 8th edition of the AJCC we found that:

Tumor size (T): 20% of the cases were of T1 stage, 20% were of T2 stage, 44% were of T3 stage & 16% were of T4 stage, considering that T1 & T2 cases show the early staged disease (40% of the cases) and T3 & T4 make a sum of (60%) representing the locally advanced form of the tumor.

Node (N): 56% showed no nodal metastasis (N0), 28 cases, 26% showed nodal metastasis as the following: N1 (7cases), N2a (10cases), N2b (5 cases).

Metastasis (M): all the patients were without evidence of distant metastasis (M0) at the time of the diagnosis.

		N	%
P 16	Positive	45	90
	Negative	5	10
P 53	Positive	39	78
	Negative	11	22
Cyclin d1	Positive	25	50
	Negative	25	50

Table 1: Demonstration of the number & percentage of p16, p53 and cyclin d1 expressions regarding the studied markers' expressions in the selected cases.

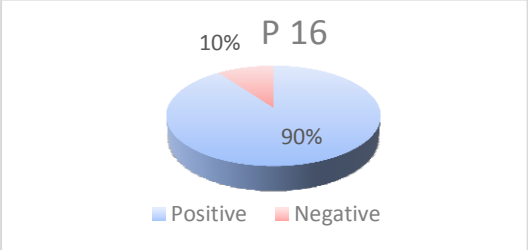

Fig 1: Illustration shows different p16 expressions'in the studied cases. This figure shows that 90% (45) cases showed positive p16 expression, while only 10% (5) cases showed negative p16 expression, thereby denotes the dominancy of p16 positivity in this study.

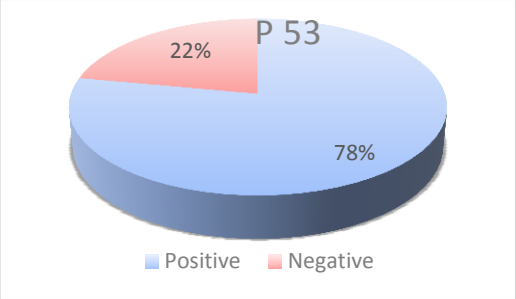

Fig 2: Illustration shows p53 expression in the studied cases. This figure shows that p53 was expressed by 78% (39) cases, while p53 expression couldn't be traced at the remaining 22% (11) cases.

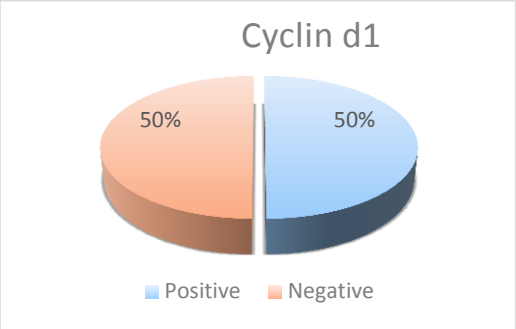

Fig 3: Illustration shows p53 expression in the studied cases. This figure shows that cyclind1 was equally expressed in 50% (25) cases as well as unexpressed in the other 50% (25) cases.

			P 16 +ve	-ve	X2	P-value
Age	Range		31 – 80	52 – 82	T: 1.989	0.052*
	Mean ± SD		58.93 ± 11.18	69.40 ± 10.99		
Gender	Male	N	41	4	0.617	0.432
		%	91.1%	80.0%		
	Female	N	4	1		
		%	8.9%	20.0%		
Smoking	+ve	N	37	3	1.389	0.239
		%	82.2%	60.0%		
	-ve	N	8	2		
		%	17.8%	40.0%		
Alcohol consumption	+ve	N	3	1	1.087	0.297
		%	6.7%	20.0%		
	-ve	N	42	4		
		%	93.3%	80.0%		
Relevant Family history	+ve	N	5	1	0.337	0.562
		%	11.1%	20.0%		
	-ve	N	40	4		
		%	88.9%	80.0%		

Table 1: Correlation between p16 expression and demographic data and the related risk factors. P16 expression showed no statistically significant correlation with neither any of the demographic data nor the related risk factors except for detecting most of the p16 expressors in the younger age group of the study (around the mean ± 11.18).

			P 16		X^2	P-value
			+ve	-ve		
Age	Range		31 – 80	52 – 82	T: 1.989	0.052*
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N.B: the mark (*) beside any p value denotes its statistical significance.

			P 16		X^2	P-value
			+ve	-ve		
Tumor Location	Supraglottic	N	16	1	4.694	0.096
		%	35.6%	20.0%		
	Glottic	N	21	1		
		%	46.7%	20.0%		
	Subglottic	N	8	3		
		%	17.8%	60.0%		
Tumor extension (Thyroid cartilage infiltration)	Present	N	24	0	5.128	0.024*
		%	53.3%	.0%		
	Absent	N	21	5		
		%	46.7%	100.0%		
Gross appearance	Ulcerating masses	N	17	1	4.694	0.096
		%	37.8%	20.0%		
	Annular infiltrating masses	N	15	1		
		%	33.3%	20.0%		
	Fungating polypoidal masses	N	13	3		
		%	28.9%	60.0%		
Histopathological types	Conventional	N	21	0	9.887	0.007*
		%	46.7%	.0%		
	Basaloid	N	13	5		
		%	28.9%	100.0%		
	Papillary	N	11	0		
		%	24.4%	.0%		

Table 3: Correlation between p16 expression & the macroscopic data and the histopathological types of the studied cases: This table shows that p16 expression developed a statistically significant correlation with the tumor extension & certain histopathological types.

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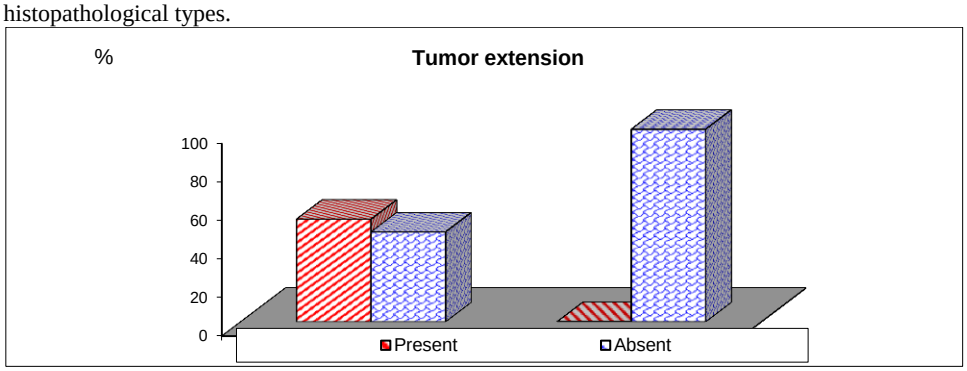

Fig 4: p16 expression in relation to tumor extension. There was a statistically significant correlation between p16 expression & tumor extension, as 24 cases (53.3%) out of 45 +ve p16 expressors showed signs of regional infiltration of the surrounding tissue, especially regional nodal infiltration, and infiltration of the underlying thyroid cartilage.

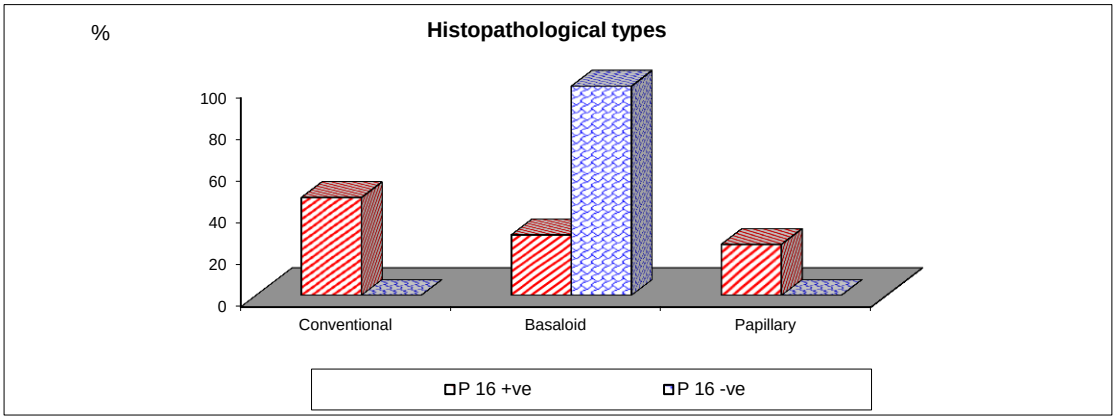

Fig 5: p16 expression regarding various histopathological types. P16 was remarkably expressed in the conventional type over the other types, as 21 cases (46.7%) out of 45 p16 expressors were of this histopathological variety, while all of the p16 non expressors 5 cases (10%) of all the studied cases were exclusively from the basaloid

			P 53		X^2	P-value
			+ve	-ve		
Tumor Location	Supraglottic	N	16	1	9.550	0.008*
		%	41.0%	9.1%		
	Glottic	N	18	4		
		%	46.2%	36.4%		
	Subglottic	N	5	6		
		%	12.8%	54.5%		
Tumor extension (Thyroid cartilage infiltration)	Present	N	24	0	13.018	0.001*
		%	61.5%	.0%		
	Absent	N	15	11		
		%	38.5%	100.0%		
Gross appearance	Ulcerating masses	N	17	1	10.988	0.001*
		%	43.6%	9.1%		
	Annular infiltrating masses	N	14	2		
		%	35.9%	18.2%		
	Fungating polypoidal masses	N	8	8		
		%	20.5%	72.7%		
Histopathological types	Conventional	N	21	0	14.240	0.001*
		%	53.8%	.0%		
	Basaloid	N	9	9		
		%	23.1%	81.8%		
	Papillary	N	9	2		
		%	23.1%	18.2%		

Table 4: Correlation between p53 expression & demographic data and related risk factors. There was no statistically significant correlation between p53 expression regarding the demographic data and related risk factors except for, age as p53 expression was found to be higher in younger cases of the study.

			P 53 +ve	-ve	X2	P-value
Tumor Location	Supraglottic	N	16	1	9.550	0.008*
		%	41.0%	9.1%		
	Glottic	N	18	4		
		%	46.2%	36.4%		
	Subglottic	N	5	6		
		%	12.8%	54.5%		
Tumor extension (Thyroid cartilage infiltration)	Present	N	24	0	13.018	0.001*
		%	61.5%	.0%		
	Absent	N	15	11		
		%	38.5%	100.0%		
Gross appearance	Ulcerating masses	N	17	1	10.988	0.001*
		%	43.6%	9.1%		
	Annular infiltrating masses	N	14	2		
		%	35.9%	18.2%		
	Fungating polypoidal masses	N	8	8		
		%	20.5%	72.7%		
Histopathological types	Conventional	N	21	0	14.240	0.001*
		%	53.8%	.0%		
	Basaloid	N	9	9		
		%	23.1%	81.8%		
	Papillary	N	9	2		
		%	23.1%	18.2%		

Table 5: Correlation between p53 expression &macroscopic data and histopathological types.P53 expression was found to be in a significant statistical correlation among the previous criteria the studied cases.

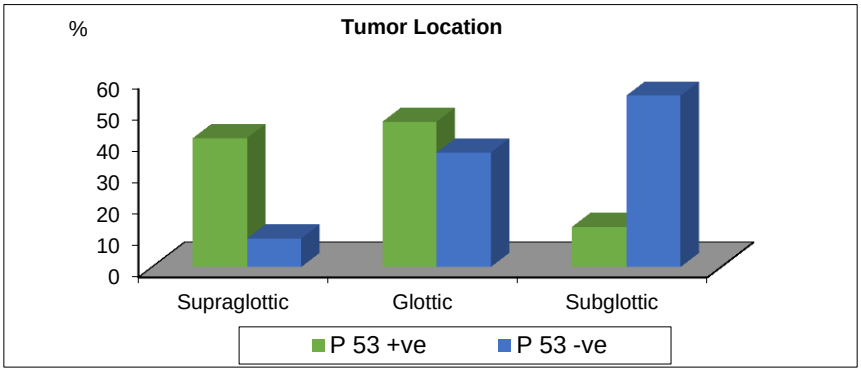

Fig 6: P53 expression in relation to tumor location. P53 expression was found to be evident in (Glottic) subvariety over the rest of cases, as 18 (46.2%) cases out of 39 p53 expressors were of glottic type, while p53 non expressors were only 11 out of 50 cases of the entire study, 6 (54.5%) cases of the non expressors were of the subglottic tumor location variety.

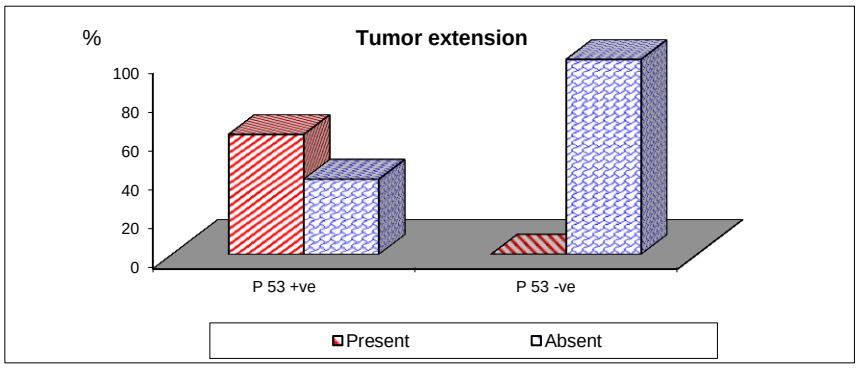

Fig 7: P53 expression in relation to tumor extension: Expression was found to be statistically significant in relation to tumor extension as 24 (61.5%) case out of 39 cases showed p53 expression & all the 11(100%) cases that didn't express p53 were constantly showing no signs of local extension nor infiltration to the surrounding tissue.

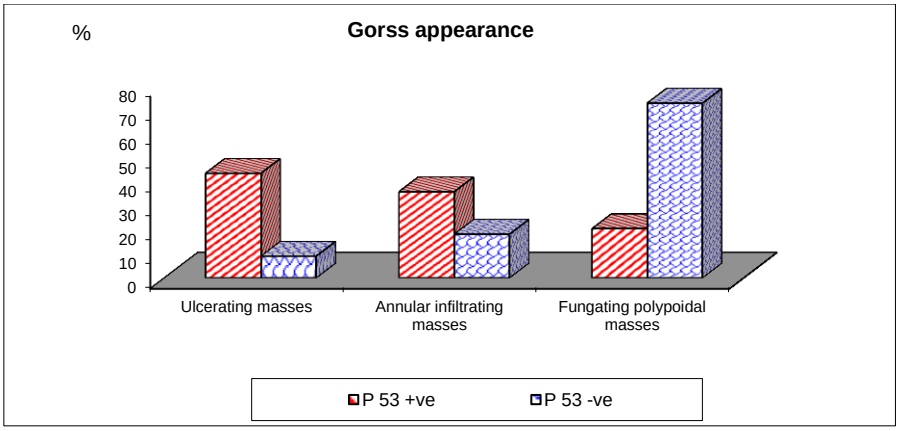

Fig 8: P53 expression in relation to gross appearance of the tumor: P53 was mostly expressed in ulcerating masses ,17(43.6%) cases out of 39 p53 expressors, while p53 was unexpressed in 11 cases ,8 (72.7%) of them were from fungating polypoidal masses type.

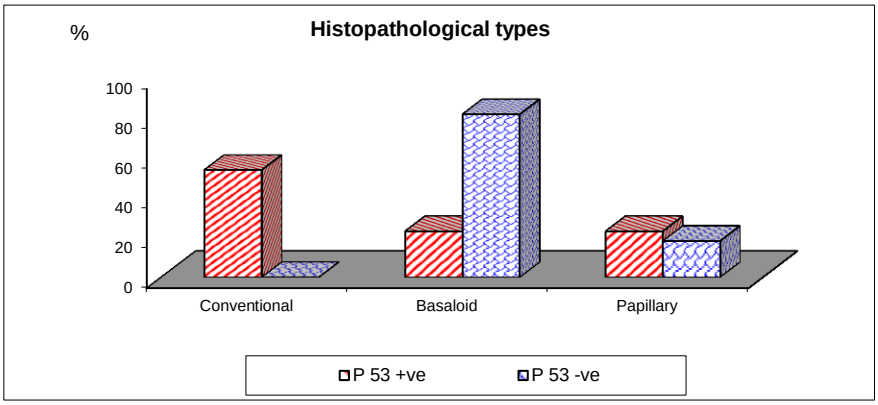

Fig 9: P53 expression in relation to various histopathological types: Giving that p53 expressors were only 39 cases, 21 (53.8%) cases out of the expressors were from the conventional histopathological type, while 11 cases showed no p53 expression 9 (81.8 %) cases out of them were of the basaloid type

			Cyclin d1 +ve	-ve	X2	P-value
Age	Range Mean ± SD		31 – 80 57.76 ± 11.21	38 – 82 62.20 ± 11.56	T: 1.378	0.175
Gender	Male	N %	23 92.0%	22 88.0%	0.222	0.637
	Female	N %	2 8.0%	3 12.0%		
Smoking	+ve	N %	22 88.0%	18 72.0%	2.000	0.157
	-ve	N %	3 12.0%	7 28.0%		
Alcohol consumption	+ve	N %	3 12.0%	1 4.0%	1.087	0.297
	-ve	N %	22 88.0%	24 96.0%		
Relevant Family history	+ve	N %	4 16.0%	2 8.0%	0.758	0.384
	-ve	N %	21 84.0%	23 92.0%		

Table 6: Correlation between cyclin d1 expression & demographic data and related risk factors. There was no statistically significant correlation between cyclin d1 expression regarding the demographic data and related risk factors.

			Cyclin d1 +ve	-ve	X2	P-value
Tumor Location	Supraglottic	N	13	4	7.219	0.027*
		%	52.0%	16.0%		
	Glottic	N	8	14		
		%	32.0%	56.0%		
	subglottic	N	4	7		
		%	16.0%	28.0%		
Tumor extension (Thyroid cartilage infiltration)	Present	N	19	5	15.705	0.001*
		%	76.0%	20.0%		
	Absent	N	6	20		
		%	24.0%	80.0%		
Gross appearance	Ulcerating masses	N	16	2	20.139	0.001*
		%	64.0%	8.0%		
	Annular infiltrating masses	N	7	9		
		%	28.0%	36.0%		
	Fungating polypoidal masses	N	2	14		
		%	8.0%	56.0%		
Histopathological types	Conventional	N	19	2	24.035	0.001*
		%	76.0%	8.0%		
	Basaloid	N	3	15		
		%	12.0%	60.0%		
	Papillary	N	3	8		
		%	12.0%	32.0%		

Table 7: Correlation between cyclin d1 expression &macroscopic data and histopathological types of the studied cases. Cyclin d1 expression was found to be in a significant statistical correlation with the previous criteria

			Cyclin d1		X^2	P-value
			+ve	-ve		
Grading (Differentiation)	**I**	**N**	2	9	26.021	0.001*
		%	8.0%	36.0%		
	II	**N**	21	3		
		%	84.0%	12.0%		
	III	**N**	2	13		
		%	8.0%	52.0%		
T stage	**T 1**	**N**	3	7	21.336	0.001*
		%	12.0%	28.0%		
	T 2	**N**	2	8		
		%	8.0%	32.0%		
	T 3	**N**	19	3		
		%	76.0%	12.0%		
	T 4	**N**	1	7		
		%	4.0%	28.0%		
N (Nodal status)	**N 0**	**N**	18	10	5.371	0.147
		%	72.0%	40.0%		
	N 1	**N**	2	5		
		%	8.0%	20.0%		
	N 2a	**N**	3	7		
		%	12.0%	28.0%		
	N 2b	**N**	2	3		
		%	8.0%	12.0%		

Table 8: Correlation between cyclin d1expression, grading and staging of the tumor in the studied cases.

Cyclin d1 expression was statistically correlated to both grading and T staging. Cyclin d1 expression was found to be remarkably evident with cases of grade II representing a significant correlation between both; 21 (84%) out of twenty-five cyclin d1 expressors were of grade II. Cyclin d1 expression was detected with cases of the stage T3 representing a significant correlation between both; 19 (76%) out of twenty-five cyclin d1 expressors were of stage T3 (Table 8).

			P 53		Total
			P16		Total
			+ve	-ve	
P 53	+ve	N	39	0	39
		%	86.7%	.0%	78.0%
	-ve	N	6	5	11
		%	13.3%	100.0%	22.0%
	Total	N	45	5	50
		%	100.0%	100.0%	100.0%
Chi-square	X^2				19.697
	P-value				0.001*

Table 9: Comparison between p16 expression and p53 expression in the studied

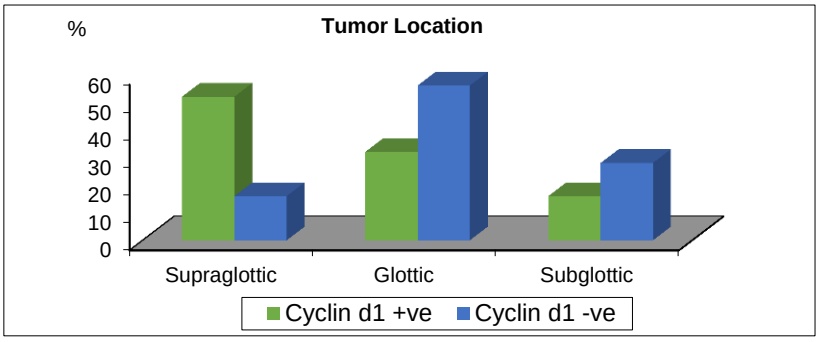

Fig 10: Cyclin d1 expression in relation to tumor location. Cyclin d1 expression showed statistically significant correlation with the tumor in the supraglottic sites;13 (52%) out of 25 cases that expressed cyclin d1, while cyclin d1 -ve cases were mostly from the glottic cases represented by 14 (56%) out of 25 cases.

			P16		Total
			+ve	-ve	
Cyclin d1	+ve	N	25	0	25
		%	55.6%	.0%	50.0%
	-ve	N	20	5	25
		%	44.4%	100.0%	50.0%
	Total	N	45	5	50
		%	100.0%	100.0%	100.0%
Chi-square	X^2				5.556
	P-value				0.018*

Table 10: Comparison between p16 expression and cyclin d1 expression in the studied cases.

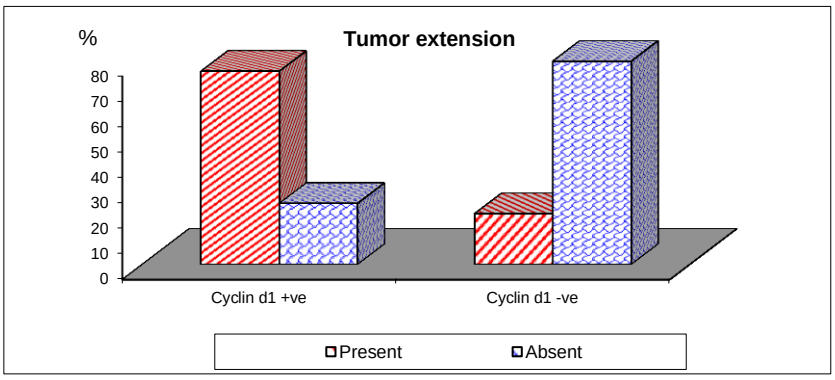

Fig 11: Cyclin d1 expression in relation to tumor extension. Significant statistical correlation was evident between cyclin d1 expression & tumor extension and vice versa, as 19 (76%) cases out of 25 cyclin expressors showed signs of tumor extension and surrounding tissues infiltration, while 20(80%) cases out of 25 cases that were cyclin cd1 -ve didn't show signs of tumor extension.

			+ve	-ve	
Cyclin d1	**+ve**	**N**	25	0	25
		%	64.1%	.0%	50.0%
	-ve	**N**	14	11	25
		%	35.9%	100.0%	50.0%
	Total	**N**	39	11	50
		%	100.0%	100.0%	100.0%
Chi-square	**X^2**				**14.103**
	P-value				**0.001***

Table 11: Comparison between p53 expression and cyclin d1expression in the studied cases.

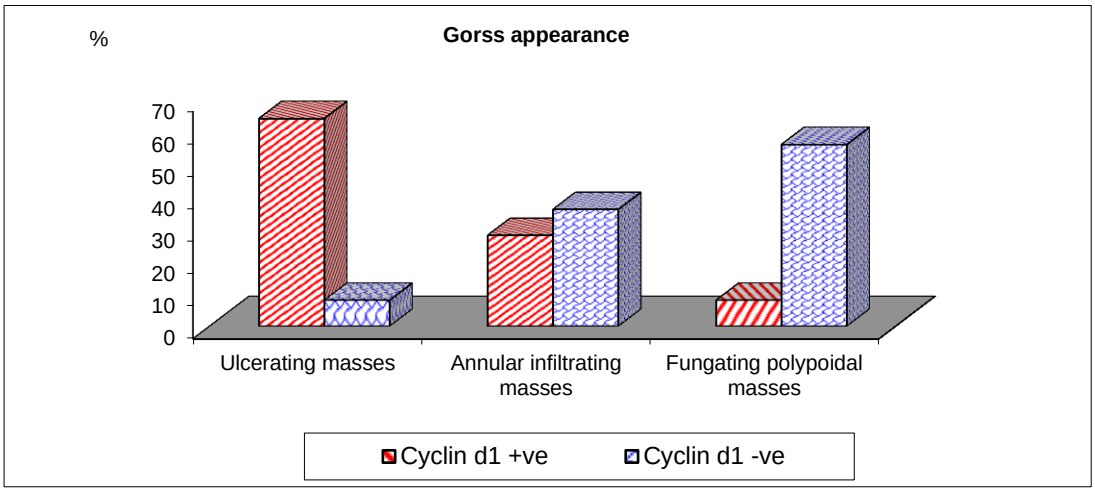

Fig 12: Cyclin d1 expression in relation to gross appearance of the tumor. Cyclin d1 expression was mostly represented in cases of ulcerating masses; 16 (64%) out of 25 cases, while among the non expressors fungating polypoidal masses took the lead by 14 (56 %) cases out of 25 as well.

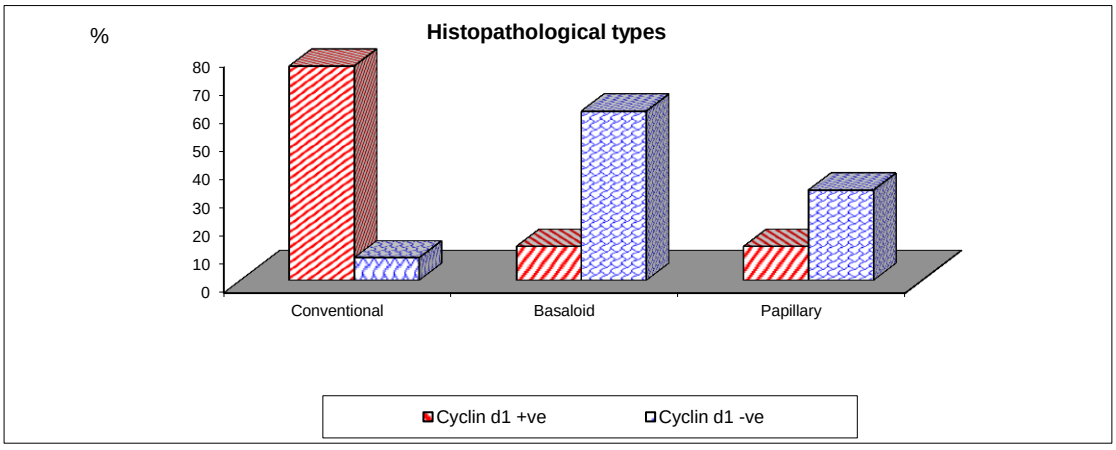

Fig 13: Cyclin d1 expression in relation to various histopathological types**.** Cyclin d1 was expressed in 25 cases 19(76%) of them were of the conventional type which represents a statistically significant correlation, on the other hand the basaloid type represented 15 (60%) cases out of the 25 cases that were cyclin d1 -ve.

DISCUSSION

Regarding the expressions of P16, P53 and cyclin d1 in the squamous cell laryngeal carcinoma samples, our results showed that 90% were positive for P16, 78% were positive for P53 and 50% were positive for cyclin d1.و Such findings agreed with the study by Zhu et al. [16] which indicated that 86% of Human papilloma virus (HPV) DNA-positive laryngeal squamous cell carci

nomas had showed overexpressed P16 and with the study done by Salama[17] on 40 Egyptian cases diagnosed with laryngeal squamous cell carcinomas, who observed positive P16 expression in 90% of LSCC cases, cyclin d1 expressors in 50% of the cases and positive p53 in 7% of cases.

On the other hand, there are controversial results reported by earlier studies regarding the expressions of P16, P53 and cyclin d1 in the squamous cell laryngeal carcinoma patients.

Some studies reported lower values of P16 in comparison with our results, as reported by Sánchez et al. [18] who indicated that only 11.38% of the laryngeal squamous cell carcinoma patients were positive for both p16 and HPV. Meshman, et al. [19] study indicated that 45.2% of laryngeal squamous cell carcinoma were p16-positive, additionally,however, Young, et al. [20] indicated that p16 expression is uncommon in LSCC.

A previous study done by Tsiambas, et al. [21] showed that p16 downregulation is a common genetic sequel in non-HPV related laryngeal cancer, which is commonly flourishes with advanced forms of the disease in this type of cancer, however, in HPV-mediated LSCCs, p16 overexpression has a close relation with a progressive and aggressive form due to upregulation of squamous intraepithelial lesions (SIL)/ laryngeal intraepithelial neoplasia (LIN) categorization.

Our results showed that p53 expression has a statistically significant correlation with the tumor location (glottic) (P value= 0.008), tumor extension (thyroid cartilage infiltration) (P value= 0.001), gross appearance (ulcerating masses) (P value= 0.001) and certain histopathological types (conventional) (P value= 0.001).

Such findings agreed with Kyrodimos, et al. [22] that showed statistical correlation of P53 regarding anatomic location (glottic), (p value =0.44).

A pilot study by Abd, et al. [23]on Egyptian patients denoted that P53 expression was connected to a high-grade tumor (P<0.001), a larger tumor about size (P=0.005), a positive nodal status (P<0.001), and a progressed tumor stage (P<0.001).

Regarding the correlation between cyclin d1 expression and the demographic data and the related risk factors, our results showed no statistically significant correlation with the demographic data and the related risk factors (P value> 0.05).

Such findings agreed with Zand, et al. [24] that demonstrated no important correlation between sex, age, smoking and drug abuse, tumor location and cyclin d1 expression in laryngeal squamous cell carcinoma patients.

Our studies showed that Cyclin d1 expression developed a statistically significant correlation with the tumor location (supraglottic) (P value= 0.027), tumor extension (thyroid cartilage infiltration) (P value= 0.001), gross appearance (ulcerating masses) (P value= 0.001) and certain histopathological types (conventional) (P value= 0.001).

Such findings agreed with a study by Yang, et al. [25] who said that the cyclin d1 expression levels directly refer to the location of the neoplasia and degree of infiltration and was related intimately to LSCC located in the supraglottic larynx.

Such findings disagreed with Zand, et al. [24] that indicated no significant correlations between Cyclin d1 expression and tumor location and tumor grade.

Our results showed that P16 expression was statistically correlated to grading (P value= 0.001) while most of the P16 expression occurred in grade II and staging (P value= 0.001) where stage 3 was the most often expressed pattern. No statistically significant correlation to the nodal status (P value> 0.05).

Such findings agreed with Salama[17], who saw a statistically important link between p16 overexpression and tumor grading.

On the contrast, a pilot study by Abd, et al. [23] on Egyptian patients wrote down that P16 expression was connected to tumor grade (P<0.001).

Our results wrote down that P53 expression was statistically correlated to grading (P value= 0.001) while P53 expression occurred in grade II repeatedly and the staging (P value= 0.003) where stage 3 was the most often expressed pattern. Regarding the nodal status, P53 expression was statistically significant with cases with no nodal metastasis (P value= 0.002).

Cercelaru, et al. [26] indicated that the higher p53 immuno-staining values were observed in high grade and advanced stage lesions.

Our results showed that cyclin d1 expression was statistically correlated to grading (P value= 0.001) while, many of the cyclin d1 expression occurred in grade II and the staging (P value= 0.003) where stage 3 was the most often expressed pattern. No statistically significant correlation to the nodal status (P value> 0.05).

Such findings agreed with Kyrodimos, et al. [22] study that showed that cyclin d1 expression was associated to stage and grade of the examined patients (p value=0.001; 0.0014, respectively).

Regarding the correlations between p16, P53 and Cyclin d1 expressions in the studied cases, our results showed that p16 expression is closely and linked to Cyclin d1 expression, p16 expression is related to P53 expression and p53 expression is closely related to Cyclin d1 expression, and all the studied markers' expressions were statistically correlated to each other.

Such findings agreed with Salama[17], who said that the presence of significant correlation between p16 overexpression on the one hand and Cyclin d1 and p53 on the other hand.

On the contrary, studies by Abd, et al. [23] and Shinohara, et al. [27] stated an inverse significant correlation between p16 and p53 expressions in laryngeal carcinoma. Additionally, Fu, et al. [28] study on Laryngeal squamous cell carcinoma patients showed a significant negative correlation between p16 immunostaining and cyclin d1 immunostaining (P = 0.011).

CONCLUSION

P16, P53 and Cyclin d1 are considered as indirect markers for Human papilloma virus (HPV) related LSCC. P16 is correlated with tumor extension and grade. P53 and Cyclin d1 are corelated with tumor extension, grade, and nodal metastasis, hence, p16,

p53 and Cyclin dl are considered as poor prognostic indicators in Laryngeal squamous cell carcinoma (LSCC).

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