Immunohistochemical Expression of Antioxidant Enzyme PRDX6 in Oral Squamous Cell Carcinoma In Relation to Clinicopathological Parameters

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Abstract

Background: The PRDX6 antioxidant family is important in the protection of cells against oxidants in humans. It is encoded by the PRDX6 gene. Peroxiredoxin 6 antioxidant enzyme (PRDX 6) has been reported in several cancers. However, the function of PRDX 6 in oral squamous cell carcinoma (OSCC),is far from being understood.

Objective: To normal corresponding oral mucosa in relation to clinicopathological parameters.

Patients and Methods: This study included 40 formalin-fixed, paraffin-embedded tissue blocks of primary OSCC cases and fifteen normal oral mucosa samples. Demographic data, clinical staging and histopathological grading were obtained. Immunohistochemically staining anti PRDX 6 antibody was done to illustrate its subcellular localization in normal and malignant cells.

Results: Females were affected more than males and the tongue was the most common site involved. PRDX 6 expression was high in OSCC than normal (p=0.001) with mean±Sd (60±18.8) (23±9.8). Significant correlations were found with tumor size, lymph node and tumor stage (p=0.008, p=0.012, p=0.011 respectively) . Whereas no relation was observed concerning age, gender and tumor site.

Conclusion: Significant correlation of PRXD 6 anti oxidant enzyme expression with tumor size, lymph node involvement and clinical staging, suggesting its role in oral squamous cell carcinoma progression.

Key words: Oxidative stress, PRDC 6, squamous cell carcinoma.

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Introduction

Regarding malignant head and neck tumors, squamous cell carcinoma (OSCC)of the head and neck is the most common epithelial neoplasia. It is the sixth most common cancer with an annual incidence of 500,000 and represent about 90% of all oral malignancies (Salian *et al.,* 2016). The survival of OSCC patients has not significantly improved in spite of development of many biological markers (Molinolo *et al.,* 2009). Clinicopathological criteria are important for the management and prediction of outcome of patients. However, within a group of patients sharing

the same features, there is still significant variation in prognosis (Thomas *et al.,* 2005). Oxidative stress is a serious imbalance between oxidation and antioxidants, however, it builds on definitions about oxidation, antioxidants, and balance (Sies, 2015). The definition of oxidation seems also simple: loss of electrons by a species, gain of oxygen, or loss of hydrogen. However, if something is oxidized, something else must be reduced. In biology, there is a pecking order of oxidants.

An antioxidant is any substance that, when present at low concentrations as compared with those of an oxidizable substrate, significantly delays or prevents the oxidation of that substrate (Azzi et al., 2004; Halliwell, 2004;Levonen et al., 2014). The chemical term an antioxidant is clearly different from a reducing agent. A reducing agent may even be a prooxidant if it reduces oxygen to free radicals or converts transition metal ions to lower oxidation states that react more readily with peroxides. Many biological reducing agents can be anti- or prooxidants, depending on the levels of O2 and transition metal ions around (Sies, 2015).

The Peroxiredoxin (PRDX) antioxidant protein family is preseny in a high range of species and protect cells against oxidants, in DNA is encoded by the PRDX6 gene. It is a part of the peroxiredoxin family of antioxidant enzymes (Cong et al., 2013). The crystal structures of six PRDXs in mammals have been published in 1998, including four distinctive 2-Cys PRDXs (PRDX1, PRDX1I, TryP and AhpC), one atypical 2-Cys PRDX (PRDX V) and one 1-Cys PRDX (PRDX6). PRDX2, 3, 5 and 6 planes were shown to be increased in malignant mesothelioma, in other hand PRDX2 and 3 were overexpressed in breast malignancy specimens compared with no malignant tissues (Kinnula et al., 2002; Lee et al., 2015).

The PRDXs are antioxidants, so they support survival and tumor maintenance by covering cells from oxidative stress-induced apoptosis (Neumann et al., 2007). In a study on ovarian cancer, high expression of PRDX 6 attenuates cisplatin- act to apoptosis in human ovarian malignancy cells (Pak et al., 2011). In other side, reduction of PRDX6 expression increased peroxide-induced cell death in liver cancer cells (Walsh et al., 2009). The invasion and metastasis promoting actions of PRDX6 has been found in lung cancer cells through activation of Akt via activation of phosphoinositiede 3-kinase (PI3K) and p38 kinase (Lee et al., 2009; Ho et al., 2010). The activity of PRDX6 contributes to the metastatic ability of lung cancer cells by stimulating invasion components including PI3K, Akt, and uPA (Ho et al., 2010). It was also reported that PRDX6 expression in lung cancer cells was significantly associated with tumor progression (Schremmer et al., 2007).

Jhang et al., in 2010 approve that targeting PRDX6 may sensitize cancer cells to ROS-producing therapeutic treatments, such as anticancer drugs and radiation (Jhang et al., 2011). In 2013, Rolfs reported that, the potential usefulness of PRDX6 activators or

inhibitors for controlling different stages of skin carcinogenesis (Rolfs *et al.,* 2013). Other recent research showed that peroxiredoxin 6 can be regarded as a prophylactic radioprotective agent (Sharapov *et al.,* 2016). The immune marker Anti-Peroxiredoxin 6 antibody is Rabbit monoclonal to Peroxiredoxin 6, belongs to the ahpC/TSA family. Rehydrin subfamily. Contains 1 thioredoxin domain and which have Predicted molecular weight 25 kDa.

The present study investigated the expression levels of PRDX 6 in OSCC in comparison to normal oral mucosa, which have previously been found to be highly expressed in various malignant neoplasms. Thus, the valuable predictive factors of OSCC are required to prevent the tumorigenesis, progression and recurrence of the disease.

Patients and Methods

Fifty-five formalin-fixed, paraffin-embedded tissue blocks, including fifteen normal oral mucosa (alveolar mucosa obtained during orthodontic extractions or implantation process). In addition, forty primary squamous cell carcinoma surgical specimens obtained from the achieves of department of oral pathology / college of Dentistry- Baghdad University, and some private laboratories were included in this study. Data concerning patient's age, gender, site, clinical staging and histopathological grading were obtained from the associated patients' reports. Tumor histopathological sections were reviewed by two pathologists, and the representative paraffin blocks were

selected. A section of 4-μm thickness was cut from each tissue block and mounted on positively charged slides (Esco, USA) to be stained with monoclonal antibodies to PRDX6 antioxidant marker (Abcam ab133348 UK). Negative and positive tissue controls were included into each immunohistochemical run.

The slides were baked in hot air oven at 65°C overnight. Sections were sequentially de waxed through a series of xylene, graded alcohol and water immersion steps. Drops of hydrogen peroxide block were added to slides were in a ready to use package (ab126738). All slides were followed by the application of the primary antibodies with a dilution of 1:250. The slides were incubated for 1 h at 37°C and then kept at 4°C in a humid chamber overnight. Next day, after washing with PBS (Phosphate Bupher Solution), biotinylated antimouse IgG were applied to the sections, incubated and rinsed with a stream of PBS. Conjugated antibodies were visualized with DAB chromogen. Sections were counterstained with Mayer's hematoxylin for 1–2 min, dehydrated and mounted, all these laboratory procedures were performed in the college of dentistry/ Laboratory of department of oral pathology.

Estimation of the immunohistochemical results: The immunostaining results were blindly understood by two self-regulating oral pathologists under the light microscope at a magnification of 400. A semi quantitative scoring scheme was used to determine the percentage of positively stained cells as brown cytoplasmic

expression. The ratio of positive immunostained cells was graded and scored as follows: 0 = no stained cells; 1 = 1–25% positive cells; 2 = 26–50% positive cells; 3 = 51–75% positive cells; and 4 = extra than 75% positive cells. (Huang et al., 2011).

Statistical Analysis

Statistical analyses were performed utilizing computer assisted SPSS version (12) (SPSS professional 2005). The parametric statistical tests were used namely two –tailed T-test and ANOVAs table. The non-parametric statistical tests were used namely Chi- square test. P-values less than (0.05) level of significance was considered statistically significant at 95% confidence. The statistical correlations were measured by Pearson's and Spearman's linear correlation coefficient.

Results

As shown in table (1), males were slightly more frequent among healthy controls group, 9 (60%). The age of healthy controls ranged between 25 to 68 years of age with a mean of 49 +/- 11 years of age. The highest category of controls were 40-59 years of age (66.7%). Regarding OSCC sample patients, females were slightly more frequent among cases group 29 (72.5%). The age of cases group ranged between 19 - 81 years of age with a mean of 54 +/- 15 years of age. In addition, the highest category of this study group were 40-59 years of age (42.5%), as shown in table (2). According to site distribution, the tongue was the most frequently reported site of tumor 16(40.0%) figure (1).

When clinical staging of the studied sample was evaluated, the tumor size was less than 2 cm in 14(35%) of cases, while it was exceptionally large (>4 cm) in 11(27.5%) of cases and invasive into adjacent area in 2(5%) of cases only. No lymph node involvement was observed in 19(47.5%) of cases, while it reached to the stage of N2 (single ipsilateral lymph node involvement 3 to 6 cm) in 5(12.5%) of cases. In about a quarter of cases 11(27.5%) the tumor was at Stage-I, while it reached to stage-IV in 6(15%) of cases only. table (3). Well differentiated OSCC comprised more than half 22(55%) of the studied cases group Figure(2).

Peroxiredoxin 6 monoclonal antibody IHC expression subcellular localization was in the cytoplasm (Figures 3,4,5,6,7). The mean percentage of positively stained cells with peroxiredoxin 6 was significantly higher (p= 0.001) in OSCC group than healthy control group with mean±Sd (60±18.8) and (23±9.8) respectively. The median score of peroxiredoxin 6 was significantly higher (p= 0.001) with (strong staining 51-74%) in OSCC group than control group (weak staining 1-25%) (Figure 8, Tables 4A,4B). Most of healthy controls were in group (40-59) years of age and all of them showed weak expression (1-25%) with a non significant statistical relations were found neither with age nor with gender (Tables 5A,5B).

A similar non significant statistical relations were found, between study group of OSCC neither with age, gende,r nor with site. Most

OSCC cases were present in the tongue that showed strong expression and the less site was labial mucosa which showed weak expression (Tables 6A,6B,6C).

The median score category of peroxiredoxin 6 was lowest (moderate staining) among cases with T1 category (smallest tumor size ≤ 2cm) and increase with higher T stage to reach its highest median score of very strong among cases with highest T stage (tumor invading adjacent area). The observed difference in median score of peroxiredoxin 6 between tumor T staging was statistically significant. Moreover, this marker showed a statistically significant moderately strong positive linear correlation with tumor T stage (r=0.543) (Table 7). In a similar context, PRDX6 had a significant relation with lymph node involvement, the observed difference in median score of peroxiredoxin 6 between tumor N staging was statistically significant. This marker showed a statistically significant moderately strong positive linear correlation with tumor N stage (r=0.474) (Table 8). As shown in table 9, the median score category of peroxiredoxin 6 was lowest (moderate staining) among cases with tumors in the early stage (Stage-I) and progressively increase in magnitude of marker score to reach its highest value (very strong staining category score) among OSCC cases with highest tumor stage (Stage-IV). The observed difference in median score of peroxiredoxin 6

among the four tumor stages was statistically significant. Also, this marker showed a statistically significant moderately strong positive linear correlation with tumor N stage (r=0.455).

The peroxiredoxin 6 was associated with a high validity in differentiating between OSCC cases and healthy controls, since its ROC area was high (0.94), which would provide an excellent test, diagnosing OSCC separating it from a healthy person (Table 10). As shown in table 12, the optimum cut-off value for peroxiredoxin 6 score when used as a test to diagnose OSCC differentiating it from healthy controls is moderate or higher score, since it was associated with the highest accuracy (90.7%). In addition, this cut-off value is also the one associated with highest sensitivity (95%). The confidence in a positive diagnosis (real presence of OSCC) would increase to 97.7% in a clinical context where having the tumor is highly probable on other clinical evidence (pretest probability = 90%). Raising the cut-off value to the strong score or higher provides a test marker of perfect specificity (100%). Testing positive at this highly specific cut-off value (obtaining a marker score of strong or very strong category) would establish a possible diagnosis of OSCC with 100% confidence in any clinical situation.

Table (1): Frequency distribution of healthy controls group by selected variables.

		N	%
1.	Gender		
	Female	6	40.0
	Male	9	60.0
	Total	15	100.0
2.	Age (years)		
	<40	3	20.0
	40-59	10	66.7
	60+	2	13.3
	Total	15	100.0

Table (2): Frequency distribution of cases group (OSCC) by selected variables.

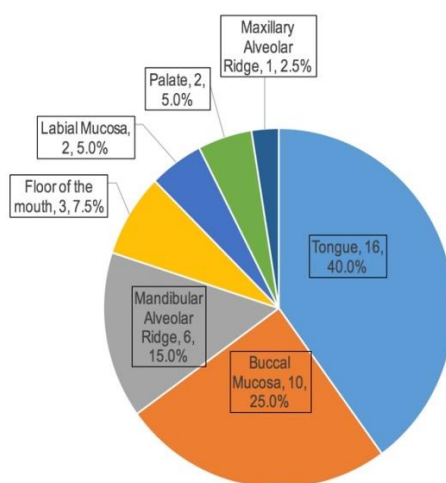|		N	%
1.	Gender		
	Female	29	72.5
	Male	11	27.5
	Total	40	100.0
2.	Age (years)		
	<40	7	17.5
	``	17	42.5
	60+	16	40.0
	Total	40	100.0

Figure (1): Pie chart showing the relative frequency of selected tumor locations (N=40).

Table (3): Frequency distribution of cases group (OSCC) by selected variables.

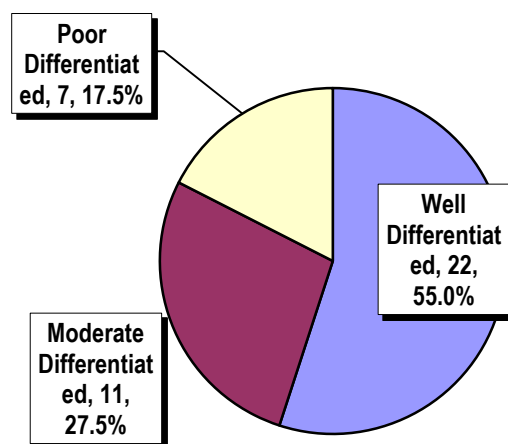|		N	%
1.	T (Tumor Size)		
	≤ 2 cm	14	35.0
	2 - 4 cm	13	32.5
	> 4 cm	11	27.5
	Invasive to adjacent area	2	5.0
	Total	40	100.0
2.	N (lymph Node involvement)		
	No LN involvement	19	47.5
	N1 metastasis to single ipsilateral LN ≤ 3 cm	16	40.0
	N2 metastasis to single ipsilateral LN 3 - 6 cm	5	12.5
	Total	40	100.0
3.	Tumor stage		
	Stage I	11	27.5
	Stage II	6	15.0
	Stage III	17	42.5
	Stage IV	6	15.0
	Total	40	100.0

Figure (2): Pie chart showing the relative frequency of cases group by degree of tumor
differentiation (N=40).

Figure (3): Photomicrograph showing Positive brown cytoplasmic expression of PRDX6 A
immunostaining in Human brain (Positive Control) (Original magnification X400).

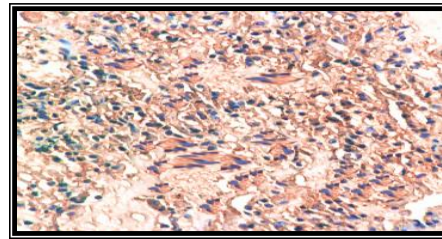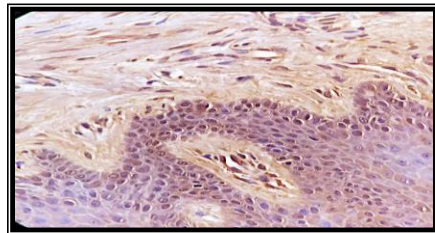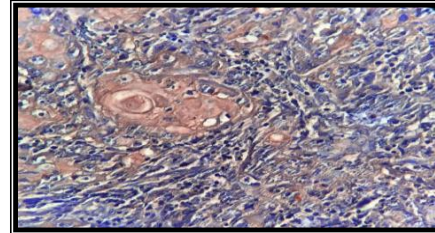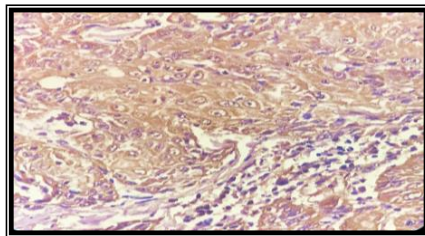**Figure(4):**Photomicrograph showing Positive brown cytoplasmic expression of peroxiredoxin 6 in normal oral mucosa (Original magnification X400).

Figure(5): Photomicrograph showing Positive brown cytoplasmic expression of peroxiredoxin 6 in well differentiated OSCC (Original magnification).

Figure(6): Photomicrograph showing Positive brown cytoplasmic expression of peroxiredoxin 6 in moderately differentiated OSCC (Original magnification X400).
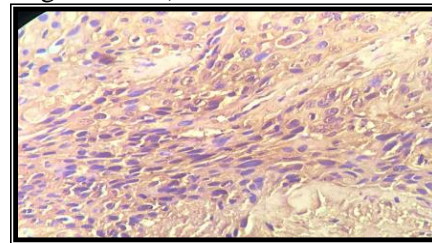

Figure(7): Photomicrograph showing Positive brown cytoplasmic expression of peroxiredoxin 6 in poorly differentiated OSCC (Original magnification X400).
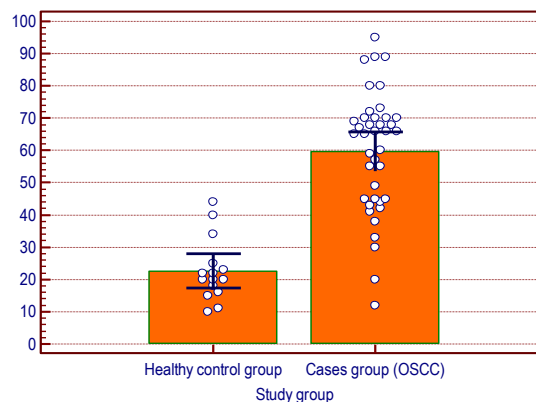

Figure (8): Dot diagram with error bars showing the case-control difference in mean (with its 95% confidence interval) peroxiredoxin 6 percent positive cells.

Table (4A): Case-control difference in mean percentage of cells positive for selected markers.

	Study group		
	Healthy control group	Cases group (OSCC)	P
peroxiredoxin 6-percent positive cell			<0.001
Range	(10 to 44)	(12 to 95)	
Mean	23	60	
SD	9.8	18.8	
SE	2.5	3	
N	15	40	

* NS Non-significant relation (p> 0.0)

Table (4B): Case-control difference in median value of selected marker scores.

	Study group				
	Healthy control group		Cases group (OSCC)		
	N	%	N	%	P
peroxiredoxin 6 score					<0.001
Negative (0%)	0	0.0	0	0.0	
Weak (1-25%)	12	80.0	2	5.0	
Moderate (26-50%)	3	20.0	11	27.5	
Strong (51-75%)	0	0.0	21	52.5	
Very strong (76-100%)	0	0.0	6	15.0	
Total	15	100.0	40	100.0	
Median	Weak (1-25%)		Strong (51-75%)		
Mean Rank	10.3		34.6		

* NS Non-significant relation (p> 0.05)

Table (5A): The median value of selected marker scores by age group among healthy controls.

	Age (years)						
	<40		40-59		60+		
	N	%	N	%	N	%	P
peroxiredoxin 6-score							0.68[NS]
Negative (0%)	0	0	0	0	0	0	
Weak (1-25%)	2	66.7	8	80	2	100	
Moderate (26-50%)	1	33.3	2	20	0	0	
Strong (51-75%)	0	0	0	0	0	0	
Very strong (76-100%)	0	0	0	0	0	0	
Total	3	100	10	100	2	100	
Median	Weak (1-25%)		Weak (1-25%)		Weak (1-25%)		
Mean Rank	9		8		6.5		
r=-0.21 P=0.45[NS]							

* NS Non-significant relation (p> 0.05)

Table (5B): The median value of selected marker scores by gender among healthy controls.

	Gender				
	Female		Male		
	N	%	N	%	P
peroxiredoxin 6-score					0.8[NS]
Negative (0%)	0	0	0	0	
Weak (1-25%)	5	83.3	7	77.8	
Moderate (26-50%)	1	16.7	2	22.2	
Strong (51-75%)	0	0	0	0	
Very strong (76-100%)	0	0	0	0	
Total	6	100	9	100	
Median	Weak (1-25%)		Weak (1-25%)		
Mean Rank	7.8		8.2		

* NS Non-significant relation (p> 0.05)

Table (6A): The median value of selected marker scores by age group among OSCC cases group.

	Age (years)						
	<40		40-59		60+		
	N	%	N	%	N	%	P
peroxiredoxin 6-score							0.44[NS]
Negative (0%)	0	0	0	0	0	0	
Weak (1-25%)	1	14.3	0	0	1	6.3	
Moderate (26-50%)	3	42.9	4	23.5	4	25	
Strong (51-75%)	2	28.6	11	64.7	8	50	
Very strong (76-100%)	1	14.3	2	11.8	3	18.8	
Total	7	100	17	100	16	100	
Median	Moderate (26-50%)		Strong (51-75%)		Strong (51-75%)		
Mean Rank	15.9		21.8		21.1		
r=0.135 P=0.41[NS]							

Table (6B): The median value of selected marker scores by gender among OSCC cases group.

	Gender				
	Female		Male		
	N	%	N	%	P
peroxiredoxin 6-score					0.37[NS]
Negative (0%)	0	0	0	0	
Weak (1-25%)	1	3.4	1	9.1	
Moderate (26-50%)	10	34.5	1	9.1	
Strong (51-75%)	14	48.3	7	63.6	
Very strong (76-100%)	4	13.8	2	18.2	
Total	29	100	11	100	
Median	Strong (51-75%)		Strong (51-75%)		
Mean Rank	19.6		23		

Table (6C): The median value of selected marker scores by site among OSCC cases.

	Tumor site														
	Tongue		Mandibular Alveolar Ridge		Floor of the mouth		Buccal Mucosa		Labial Mucosa		Maxillary Alveolar Ridge		Palate		
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	P
peroxiredoxin 6-percent positive cell-categories															0.32[NS]
Weak (1-25%)	2	11.8	3	33.3	1	20.0	2	18.2	1	33.3	3	60.0	2	40.0	
Moderate (26-50%)	4	23.5	1	11.1	1	20.0	4	36.4	0	0.0	2	40.0	2	40.0	
Strong (51-75%)	10	58.8	4	44.4	2	40.0	3	27.3	2	66.7	0	0.0	0	0.0	
Very strong (76-100%)	1	5.9	1	11.1	1	20.0	2	18.2	0	0.0	0	0.0	1	20.0	
Total	17	100.0	9	100.0	5	100.0	11	100.0	3	100.0	5	100.0	5	100.0	
Median	Strong (51-75%)		Strong (51-75%)		Strong (51-75%)		Moderate (26-50%)		Strong (51-75%)		Weak (1-25%)		Moderate (26-50%)		
Mean rank	32		28.1		31.9		29.4		28.5		13.1		22.1		

* NS Non-significant relation (p> 0.05)

Table (7): The median value of selected marker scores by tumour size among OSCC cases group.

	T (Tumor Size)								
	≤ 2 cm		2 - 4 cm		> 4 cm		Invasive to adjacent area		
	N	%	N	%	N	%	N	%	P
peroxiredoxin 6-score									0.008
Negative (0%)	0	0	0	0	0	0	0	0	
Weak (1-25%)	1	7.1	1	7.7	0	0	0	0	
Moderate (26-50%)	8	57.1	2	15.4	1	9.1	0	0	
Strong (51-75%)	5	35.7	8	61.5	7	63.6	1	50	
Very strong (76-100%)	0	0	2	15.4	3	27.3	1	50	
Total	14	100	13	100	11	100	2	100	
Median	Moderate (26-50%)		Strong (51-75%)		Strong (51-75%)		Very strong (76-100%)		
Mean Rank	13.3		21.9		26.2		30.8		
r=0.543 P<0.001									

* NS Non-significant relation (p> 0.05)

Table (8): The median value of selected marker scores by lymph Node involvement among OSCC cases group.

	N (lymph Node involvement)						
	No LN involvement		N1 metastasis to single ipsilateral LN ≤ 3 cm		N2 metastasis to single ipsilateral LN 3 - 6 cm		
	N	%	N	%	N	%	P
peroxiredoxin 6-score							0.012
Negative (0%)	0	0	0	0	0	0	
Weak (1-25%)	2	10.5	0	0	0	0	
Moderate (26-50%)	8	42.1	3	18.8	0	0	
Strong (51-75%)	8	42.1	10	62.5	3	60	
Very strong (76-100%)	1	5.3	3	18.8	2	40	
Total	19	100	16	100	5	100	
Median	Moderate (26-50%)		Strong (51-75%)		Strong (51-75%)		
Mean Rank	15.6		23.5		29.4		
r=0.474 P=0.002							

* NS Non-significant relation (p> 0.05)

Table (9): The median value of selected marker scores by tumour stage among OSCC cases group.

	Tumor stage								
	Stage-I		Stage-II		Stage-III		Stage-IV		
	N	%	N	%	N	%	N	%	P
peroxiredoxin 6-score									0.011
Negative (0%)	0	0	0	0	0	0	0	0	
Weak (1-25%)	1	9.1	0	0	1	5.9	0	0	
Moderate (26-50%)	6	54.5	1	16.7	4	23.5	0	0	
Strong (51-75%)	4	36.4	4	66.7	10	58.8	3	50	
Very strong (76-100%)	0	0	1	16.7	2	11.8	3	50	
Total	11	100	6	100	17	100	6	100	
Median	Moderate (26-50%)		Strong (51-75%)		Strong (51-75%)		Very strong (76-100%)		
Mean Rank	13.2		23.6		20.5		30.8		
r=0.455 P=0.003									

Table (10): The median value of selected marker scores by degree of tumour differentiation among cases with OSCC.

	Degree of Differentiation (Grade)						
	Well Differentiated		Moderate Differentiated		Poor Differentiated		
	N	%	N	%	N	%	P
peroxiredoxin 6-score							0.8[NS]
Negative (0%)	0	0	0	0	0	0	
Weak (1-25%)	1	4.5	0	0	1	14.3	
Moderate (26-50%)	5	22.7	4	36.4	2	28.6	
Strong (51-75%)	13	59.1	5	45.5	3	42.9	
Very strong (76-100%)	3	13.6	2	18.2	1	14.3	
Total	22	100	11	100	7	100	
Median	Strong (51-75%)		Strong (51-75%)		Strong (51-75%)		
Mean Rank	21.2		20.6		18.1		
r=-0.091 P=0.58[NS]							

Table (11): Area under ROC curve for selected marker when used as test to diagnose OSCC differentiating it from healthy controls.

	Percent positive cells		Marker score	
	AUROC	P	AUROC	P
peroxiredoxin 6	0.94	<0.001	0.94	<0.001

Table (12): Validity parameters for selected markers when used as test to diagnose OSCC differentiating it from healthy controls.

				PPV at pretest probability=		NPV at pretest probability = 10%
Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy	50%	90%	
peroxiredoxin 6-Score						
Moderate (26-50%) (Highest sensitivity and optimum cut-off value)	95.0	80.0	90.7	82.6	97.7	99.3
Strong (51-75%) (Highest specificity)	67.5	100.0	76.8	100.0	100.0	96.5

Discussion

The PRDX6 antioxidant enzyme is originate in a large range of classes and is important in the protection of cells against oxidants, in humans is encoded by the PRDX6 gene. It is a member of the PRDX family of antioxidant enzymes (Cong et al., 2013). (Neumann *et al*., 2007) had suggested that, since PRDXs are antioxidants, they support survival and tumor maintenance by protecting cells from oxidative stress-induced apoptosis. Also, it has been detected by Huang *et al*., 2011, that PRDX6 is associated with tumorigenesis in tongue SCC. In a similar manner, (Walsh *et al.,*

2009) reported that, the reduction of PRDX6 expression increased peroxide-induced cell death in liver cancer cells. In the present study, PRDX6 over expression in both, the mean cytoplasmic localization and the median score of PRDX6 were significantly higher in OSCC group than healthy control group(p= 0.001),confirming that the malignant cells, resist ROS during their high rate cellular proliferations, escaping apoptosis and allowing tumor growth. This findings agree with other researches done on OSCC cases (Huang *et al.,* 2011;Huang et al., 2013;Lee *et al.,* 2015) who reported that,

the cancer cells showed increase the PRDX6 protein expression compared to the normal corresponding cells and found to be expressed at unusually high levels in several types of cancers including breast cancer (Chang et al., 2007). (Rolfs et al., 2013) found that, PRDX6 in keratinocytes of standard human skin and in the tumor cells of squamous cell carcinomas, representing a role of Prdx6 in human skin carcinogenesis. Whereas, (Young et al., 2015), found that there was no significant difference between OSCC and normal mucosa.

In the same context, concerning other malignant conditions, elevated levels of PRDX6 have been described in breast cancer (Thongwatchara et al., 2011), in malignant mesothelioma (Kinnula et al., 2002), in bladder cancer (Quan et al., 2006), in esophageal cancer (Zhang et al., 2011), in lung (Schremmer et al., 2007), ovarian (Pylväs et al., 2010) and pancreas (Park et al., 2011) cancer, in cancer of the gingivo-buccal area (Shukla et al., 2009), and in lymphoma (Kuusisto et al., 2015).

Concerning age, in this study, most cases were in group (40-59) years of age and all of them were showed weak expression for PRDX6 and showed no important statistically significant difference in median score between the three age groups, also the expression was weak in both male and female and showed no statistically significant difference, also regarding site, the most affected region was present in the tongue with strong expression and showed no significant correlation with site

distribution and to the best of knowledge, no such results in any study were reported .

This study showed that the the median score of PRDX6 was lowest among cases with T1 category and increase significantly with higher tumor size to reach its highest median score of very strong positivity among invading tumors to the adjacent areas (p=0.008) also, this marker showed a statistically significant moderately strong positive linear correlation with tumor size (r=0.543).Theses findings suggested that as the size of tumor increase, the antioxidant system increase it activity to resist the ROS that present during tumor cells proliferation (Lee et al., 2015). (Yi et al., 2013) on their study on esophageal SCC cases reported similar results, in contrast, (Young et al., 2015) and (Huang et al., 2013) reported that, there was no relation between tumor size and PROX6 in OSCC. Elevated expression levels of PRDX6 have been associated with a more invasive phenotype and metastatic potential of breast cancer (Chang et al., 2007; Arianna et al., 2017) , and with a worse prognosis of clinically localized prostate cancer following radical prostatectomy (Raatikainen et al., 2015), such findings suggest that the PRDX6 is important during multi steps of tumor carcinogenesis including invasion (Lee et al., 2015) .

The observed difference in median score of PRDX6 between tumor lymph node involvement was statistically significant (p=0.012) with a statistically significant moderately strong positive linear correlation with tumor N stage (r=0.474).These findings

agree with (Zhong et al., 2007) in their study on breast cancer. Such results suggested that, PROX6 can be used as predictable marker for lymph node metastasis of OSCC for early treatment and need more study confirmation. Other investigators as (Zhang et al., 2012;Huang et al., 2013; Yi et al., 2013) found opposite results with the present study findings in SCC cases. Recently in a study review explained the role of PRDX6 in several tumors and the role of PRDX6 in tumorigenesis, recurrence and invasion. (Nicolussi et al., 2017) they reported that, there was no relation with lymph node involvement. The invasion and metastasis promoting actions of PRDX6 has been found in lung cancer cells through activation of Akt via activation of phosphoinositiede 3-kinase (PI3K) and p38 kinase (Lee et al., 2009;Ho et al., 2010). The activity of PRDX6 contributes to the metastatic ability of lung cancer cells by stimulating invasion components including PI3K, Akt, and uPA (Ho et al., 2010). Overexpression of PRDX6 leads to a more invasive phenotype and metastatic potential in human breast cancer, through regulation of the levels of uPAR, Ets-1, MMP-9, RhoC and TIMP-2 expression (Chang et al., 2007).

Regarding tumor stage, study findings showed that, the median score category of PRDX6 was lowest (moderate staining) among cases with tumors in the early stage (Stage-I) and progressively increase in magnitude of marker score to reach its highest value (very strong stain category score) among OSCC cases with highest tumor stage (Stage-IV) and also the observed difference in median score of PRDX6 between the four tumor stages was statistically significant. This marker showed a statistically significant moderately strong positive linear correlation with tumor N stage (r=0.455), these results confirms that PRDX6 is an important antioxidant enzyme regulating the cellular redox state in malignant cells with potential effects on tumor growth by preventing apoptosis. So PRDX6 could be of a benefit in tumor staging of OSCC in addition to the traditional methods till more researches in the future will be conducted to confirm results.

Concerning the degree of tumour differentiation, a non significant correlation between expression of PRDX6 and degree of differentiation of OSCC cases was found in this study, this result agreed with (Huang et al., 2013) and such results were same as in pancreatic cancer (Lister et al., 2011) , The exact molecular gadgets of how ROS and PRDX6 effect the carcinogenesis, however, remain sophisticated and unexplained to date, it is necessary to conduct further studies with a larger sample size to clarify the effect of this antioxidant in relation to cancer cells differentiation.

However, the molecular way between oxidant stress and the cellular redox-regulating enzymes in carcinomas have not yet been fully unstated, as well as the opposing reported significance of ROS and antioxidant systems within the carcinogenesis need more tests.

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