PTEN and α-SMA Expression and Diagnostic Role in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma with Concomitant Oral Submucous Fibrosis

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ABSTRACT

Objectives: The diagnostic role and correlation between phosphatase and tensin homologue and alpha-smooth muscle actin in oral submucous fibrosis and oral squamous cell carcinoma with concomitant oral submucous fibrosis was analysed by this case control study. The mechanism by which phosphatase and tensin homologue controls myofibroblast expression was also evaluated.

Material and Methods: Overall, 10 normal mucosa, 30 oral submucous fibrosis (OSF) and 30 oral squamous cell carcinoma (OSCC) with OSF were stained immunohistochemically with phosphatase and tensin homologue (PTEN) and alpha-smooth muscle actin (α-SMA). Percentage positivity, pattern of expression was statistically compared using Pearson's Chi-square and Fischer exact tests. The correlation between markers was analysed using Spearman correlation.

Results: OSF and OSCC affected males predominantly with majority below 40 years and above 40 years of age respectively. Percentage of PTEN positive cells was statistically significant with gender ($P = 0.024$) and α-SMA distribution of pattern showed a significant correlation with habits ($P = 0.018$). A significant decrease in nuclear PTEN positivity ($P < 0.001$) and a gradual increase in α-SMA cytoplasmic expression was noted from NM to OSF and OSCC. A statistically significant weak inverse correlation existed between PTEN and α-SMA.

Conclusions: A reduced phosphatase and tensin homologue expression in oral submucous fibrosis makes it more prone for malignant transformation. An increase in stromal desmoplasia modifies differentiation, invasive and proliferative capacity of tumour cells. As phosphatase and tensin homologue functions through P-Akt pathway, P-Akt with phosphatase and tensin homologue could be a therapeutic target.

Keywords: immunohistochemistry; myofibroblast; oral cancer; oral submucous fibrosis; prognosis; tumor suppressor gene.

INTRODUCTION

Oral potentially malignant disorders (OPMDs) are precursors of oral squamous cell carcinoma (OSCC). Thus prevention and early detection of OPMDs aids in decreasing the incidence and improves survival of those who could develop OSCC. Oral submucous fibrosis (OSF), a PMD has high prevalence in Indian subcontinent and South East Asia and is associated with chewing betel quid and commercially available areca nut preparations [1]. OSF is a chronic, insidious oral mucosal condition, characterized by juxtaepithelial inflammation and submucosal fibrosis induced by alkaloids (arecoline, arecaidine, guvacaine, guvacoline) and flavonoids in areca nut [2]. The younger population is mainly affected. A high malignant transformation rate ranging from 7 to 13%, and in India, ranging from 2.6 to 7.6% has been reported [1].

Tremendous efforts have been made to identify predictable diagnostic biomarkers like p53, β catenin, cyclin D1, Rb protein, Ki67 (MIB), bcl2, bax, c-met, alpha-smooth muscle actin (α-SMA), phosphatase and tensin homologue (PTEN), in OSF and their role in malignant transformation [3-5].

PTEN acts as a tumour suppressor gene and controller of myofibroblast differentiation by negatively regulating phosphatidyl inositol-3-kinase/AKT (PI3K/AKT) pathway in fibrotic disorders and malignancies of thyroid, kidney, lung, breast; endometrial precancer and also plays a role in immunity [6-11]. In OSF, like other fibrotic lesions, myofibroblasts express α-SMA and are the primary producers of extracellular matrix (ECM) [12,13]. An inverse correlation between PTEN and α-SMA expression has been noted in dermal fibrosis, idiopathic pulmonary fibrosis (IPF) and hepatic fibrosis [6,14-16].

The mechanism by which PTEN controls myofibroblast expression and the diagnostic role of PTEN and α-SMA in malignant transformation of OSF has not yet been studied. A better understanding of the relation between PTEN and α-SMA may help in unveiling their diagnostic and prognostic role in OSF and OSCC. Also the drugs targeting PI3K might have significant therapeutic activity in OSF and its malignant transformation. The presented retrospective case control study aimed at evaluating the phosphatase and tensin homologue mechanism controlling myofibroblast expression. Also analysed the diagnostic role and correlation between phosphatase and tensin homologue and alpha-smooth muscle actin in oral submucous fibrosis and oral squamous cell carcinoma with concomitant oral submucous fibrosis.

MATERIAL AND METHODS

The case control study was approved by the Institutional Review Board (IRB NO. IRB/2017/P/OP/55) of SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India. Following the approval, 70 formalin-fixed paraffin-embedded tissue blocks of OSF and OSCC with concomitant OSF having complete case details were selected randomly and retrieved from the archives of the Department of Oral Pathology and Microbiology, SDM College of Dental Sciences and Hospital. The study was conducted between January 2018 to June 2019.

The sample comprising of 70 cases was divided into three groups. The control group had 10 normal mucosa tissues from patients with no relevant habit history, with a healthy mucosa and without any systemic diseases or malignancies. The other two groups had 30 cases each of clinically and histologically (stained with haematoxylin and eosin) proven OSF and OSCC with concomitant OSF respectively. Clinical staging of OSF cases was done based on mouth opening according to Ranganathan et al. [17] and TNM staging was followed for OSCC with concomitant OSF [18]. Histopathologically, OSF was subdivided according to Roobans' grading [19] and OSCC with concomitant OSF using Broders' grading [20]. Patients with recurrence and those under treatment for oral carcinoma were excluded. The clinicopathological data of all cases were recorded and tabulated.

Tissue sections of 3 μm obtained from all cases on silane coated slides were subjected to immunohistochemical (IHC) analysis using PTEN protein rabbit monoclonal antibody (clone SP218, IgG immunoglobulin) and α-SMA mouse monoclonal antibody (clone 1A4, IgG2a immunoglobulin), and visualized by supersensitive polymer-HRP detection system (BioGenex Laboratories Inc.; San Ramon, California, USA); chromogen used was DAB (diaminobenzidine). Antigen heat retrieval was done using pressure cooker (0.1 M citrate buffer; pH 6). Standardized procedure according to the guidelines given by BioGenex Laboratories Inc. was followed. Brown precipitate in the nucleus and cytoplasm was considered to be a positive PTEN and α-SMA expression respectively.

The sections were examined at an ocular magnification of x10 and then a representative field (even staining) was chosen viewing at original magnification x40. Positive cells were counted from among 500 total tumour cells.

The following parameters were analysed:

- Percentage of positive cells: 0 = no positive cells, 1 = 1 to 25%, 2 = 26 to 50%, 3 = >50%.
- Pattern of expression: 0 = absent, 1 = membrane, 2 = cytoplasm, 3 = membrane and cytoplasm, 4 = nuclear.
- Expression in layers of mucosa: 0 = absent, 1 = basal/parabasal, 2 = spinous, 3 = superficial layer.

Statistical analysis

Non-parametric statistical analysis was done depending on the results by means of SPSS Statistics software, version 20.0 (IBM; New York, USA). Pearson's Chi-square and Fischer exact tests were used to analyse the correlation between the clinicopathological parameters, compare the IHC expression of PTEN and α-SMA and to correlate the expression with the clinicopathological parameters. Statistical significance level was defined at P < 0.05. Spearman correlation was used to find the correlation between the two markers.

RESULTS

The clinicopathological parameters of the study groups were assessed. Greater number of patients were below 40 years of age in OSF (73.3%), in contrast 63.3% cases of OSCC, were above the age of 40 years (P = 0.005). OSF group had all male patients (100%) and a male predilection was seen in OSCC (90%).

Chewing smokeless tobacco for more than 5 years was more prevalent among the groups (OSF = 93.3% and OSCC = 86.65%) than smoking.

Predominant site of involvement for OSCC was buccal mucosa (63.3%) followed by other sites- lip, alveolus (33.3%); tongue being the least affected (3.3%) (P < 0.001).

In OSF, stage I and II were combined (26.6% cases), stage III and IV were combined (73.3% cases). Similarly, in OSCC, stage I and II were combined (90% cases), stage III and IV were combined (10% cases) (P < 0.001).

Roobans histopathological grading of OSF showed most cases in the combined grade I and II (73.3%) followed by the combined grade III and IV (26.6%). The well and moderately differentiated OSCC had 76.6% and 23.3% cases respectively (P = 0.003).

In OSCC, depth of invasion (DOI) of tumour was grouped as < 5 mm (30%), 5 to 10 mm (40%) and > 10 mm (30%). Lymph node positivity was seen in 36.6% cases.

Statistical significant difference in the age, site, staging and grading among the study groups existed (Table 1).

Table 1. Clinicopathological data of groups using Pearson's Chi-square and Fisher exact test

Parameters	Category	NM-10 N (%)	OSF-30 N (%)	OSCC + OSF-30 N (%)	P-value
Age (years)	< 40	8 (80)	22 (73.3)	11 (36.6)	0.005[b]
	≥ 40	2 (20)	8 (26.6)	19 (63.3)	
Sex	Male	8 (80)	30 (100)	27 (90)	0.72
	Female	2 (20)	0	3 (10)	
Habits	Chewing	-	28 (93.3)	26 (86.6)	0.548
	Mixed	-	2 (6.7)	2 (6.6)	
Frequency	< 5 times	-	17 (56.6)	20 (66.6)	0.426
	≥ 5 times	-	13 (43.3)	10 (33.3)	
Duration	< 5 years	-	14 (46.6)	11 (36.6)	0.432
	≥ 5 years	-	16 (53.3)	19 (63.3)	
Site	Buccal mucosa	-	-	19 (63.3)	< 0.001[b]
	Others	-	-	10 (33.3)	
OSF staging	Stage I and II	-	9 (30)	-	0.11
	Stage III and IV	-	21 (70)	-	
OSF grading	Stage I and II	-	22 (73.3)	-	< 0.001[b]
	Stage III and IV	-	8 (26.6)	-	
OSCC staging	Stage I and II	-	-	27 (90)	0.028[a]
	Stage III and IV	-	-	3 (10)	
OSCC grading	Well diff	-	-	23 (76.6)	0.003[b]
	Moderately diff	-	-	7 (23.3)	
DOI	< 5 mm	-	-	9 (30)	0.741
	5 to 10 mm	-	-	12 (40)	
	> 10 mm	-	-	9 (30)	
Lymph node	Positive	-	-	11 (36.6)	0.144
	Negative	-	-	19 (63.3)	

[a]Statistically significant at level P < 0.05, Pearson's Chi-square and at level P < 0.05, Fischer exact test.
[b]Statistically highly significant at level P < 0.05, Pearson's Chi-square and at level P < 0.05, Fischer exact test.
NM = normal mucosa; OSF = oral submucous fibrosis; OSCC = oral squamous cell carcinoma; N = number.

Percentage of PTEN positive cells was statistically significant with gender (P = 0.024) and α-SMA distribution of pattern showed a significant correlation with habits (P = 0.018). A significant decrease in nuclear PTEN positivity (P < 0.001) and gradual increase in α-SMA cytoplasmic expression was noted from normal mucosa to OSF and OSCC. A statistically significant weak inverse correlation existed between PTEN and α-SMA.

PTEN nuclear expression was seen in basal and parabasal layers of normal and OSF epithelium (Figure 1) and in OSCC, in the peripheral cells of tumour islands (Figure 2) (P = 0.233). In the normal mucosa, 1 to 25% positive cells were seen in 40% and 26 to 50% in 50% of tissues, while in OSF, 66.6% and in OSCC,

30% had 1 to 25% positive cells (P < 0.001). Thus a reduction in the expression was noted i.e., 33.3% OSF cases and 70% OSCC cases failed to show any expression. This difference was statistically significant (P = 0.001) (Table 2).

In case of α-SMA, cytoplasmic positivity was seen in 30% of NM, 53.3% of OSF (Figure 3) and 56.6% of OSCC (Figure 4). There was no statistically significant difference among the study groups.

Comparison of percentage of positive cells of PTEN with the clinicopathological parameters showed a statistical significant difference for gender (P = 0.024). Distribution of pattern of PTEN showed no statistically significant difference for any of the clinicopathological parameters (Table 3).
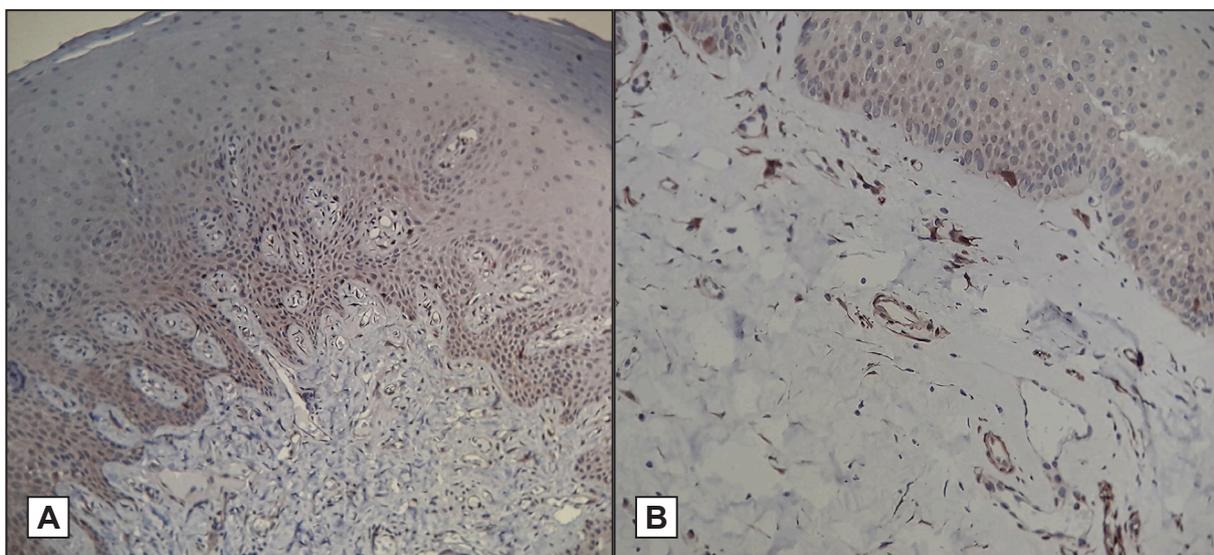

Figure 1. Nuclear phosphatase and tensin homologue staining in basal and parabasal layers.
A = normal mucosa (original magnification x20). B = oral submucous fibrosis (original magnification x40).
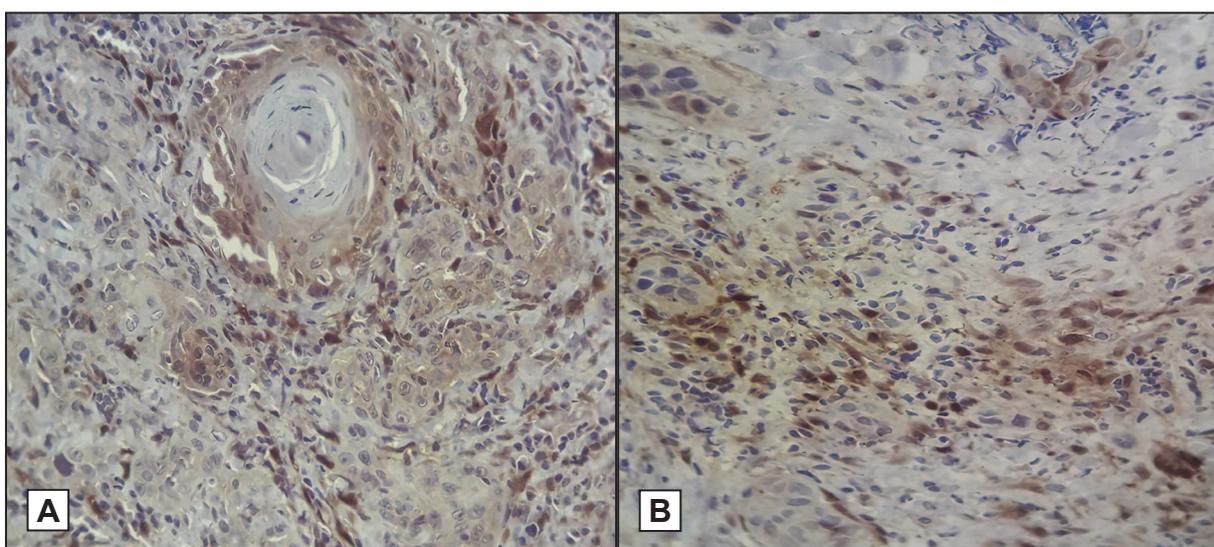

Figure 2. Phosphatase and tensin homologue (PTEN) nuclear staining.
A = Intense PTEN staining in tumour islands of oral squamous cell carcinoma (OSCC) with concomitant oral submucous fibrosis (OSF) (original magnification x20). B = Intense nuclear PTEN staining in tumour cells of OSCC with concomitant OSF (original magnification x40).

Table 2. Comparison of IHC expression of PTEN among groups using Pearson's Chi-square and Fisher exact test

Parameters	Category	NM N (%)	OSF N (%)	OSF + OSCC N (%)	P-value
Percentage positivity	0 = no positive cells	1 (10)	10 (33.3)	21 (70)	< 0.001[a]
	1 = 0 to 25%	4 (40)	20 (66.6)	9 (30)	
	2 = 26 to 50%	5 (50)	-	-	
	3 = > 50%	-	-	-	
Pattern	Absent	1 (10)	10 (33.3)	21 (70)	0.001[a]
	Cytoplasm	-	-	-	
	Nuclear	9 (90)	20 (66.6)	9 (30)	
Layers	0 = absent	1 (10)	10 (33.3)	-	0.233
	1 = basal/parabasal	9 (90)	20 (66.6)	-	
	2 = spinous	-	-	-	
	3 = superficial layer	-	-	-	

[a]Statistically significant at level P < 0.05, Pearson's Chi-square and at level P < 0.05, Fischer exact test.
NM = normal mucosa; OSF = oral submucous fibrosis; OSCC = oral squamous cell carcinoma; N = number; IHC = immunohistochemical; PTEN = phosphatase and tensin homologue.

Figure 3. Alpha-smooth muscle actin staining around the vessel walls taken as internal control and in the myofibroblast cytoplasm in the connective tissue stroma of oral submucous fibrosis (original magnification x40).
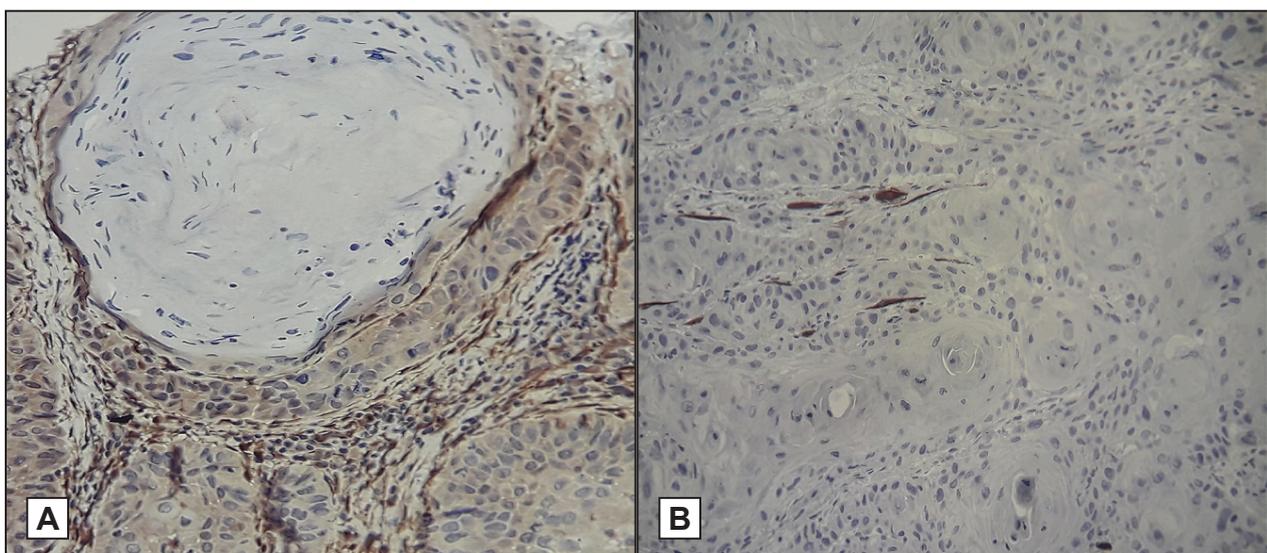

Figure 4. Cytoplasmic alpha-smooth muscle actin staining myofibroblasts in the connective tissue stroma of oral squamous cell carcinoma: A = original magnification x20; B = original magnification x40.

Table 3. Comparison of IHC expression of α-SMA among groups using Pearson Chi-square and Fisher exact test

Parameters	Category	NM	OSF	OSF + OSCC	P-value
		N (%)	N (%)	N (%)	
Percentage positivity	0 = no positive cells	7 (70)	14 (46.6)	13 (43.3)	0.426
	1 = 0 to 25%	3 (30)	16 (53.3)	15 (50)	
	2 = 26 to 50%	-	-	2 (6.6)	
	3 = > 50%	-	-	-	
Pattern	Absent	7 (70)	14 (46.6)	13 (43.3)	0.331
	Cytoplasm	3 (30)	16 (53.3)	17 (56.6)	
	Nuclear	-	-	-	

NM = normal mucosa; OSF = oral submucous fibrosis; OSCC = oral squamous cell carcinoma; N = number;
IHC = immunohistochemical; α-SMA = alpha- smooth muscle actin.

Percentage positivity of α-SMA showed no statistically significant difference for clinicopathological parameters, but distribution of pattern of α-SMA showed a significant correlation with habits.

Comparison of percentage positivity of PTEN and α-SMA showed no statistical significant difference in OSF and OSCC groups, while the distribution of pattern of PTEN and α-SMA had a high statistical significant difference in all three study groups (P < 0.001) (Figure 5).

Spearman correlation between PTEN and α-SMA among study groups showed a statistically significant weak inverse correlation (P = 0.012)
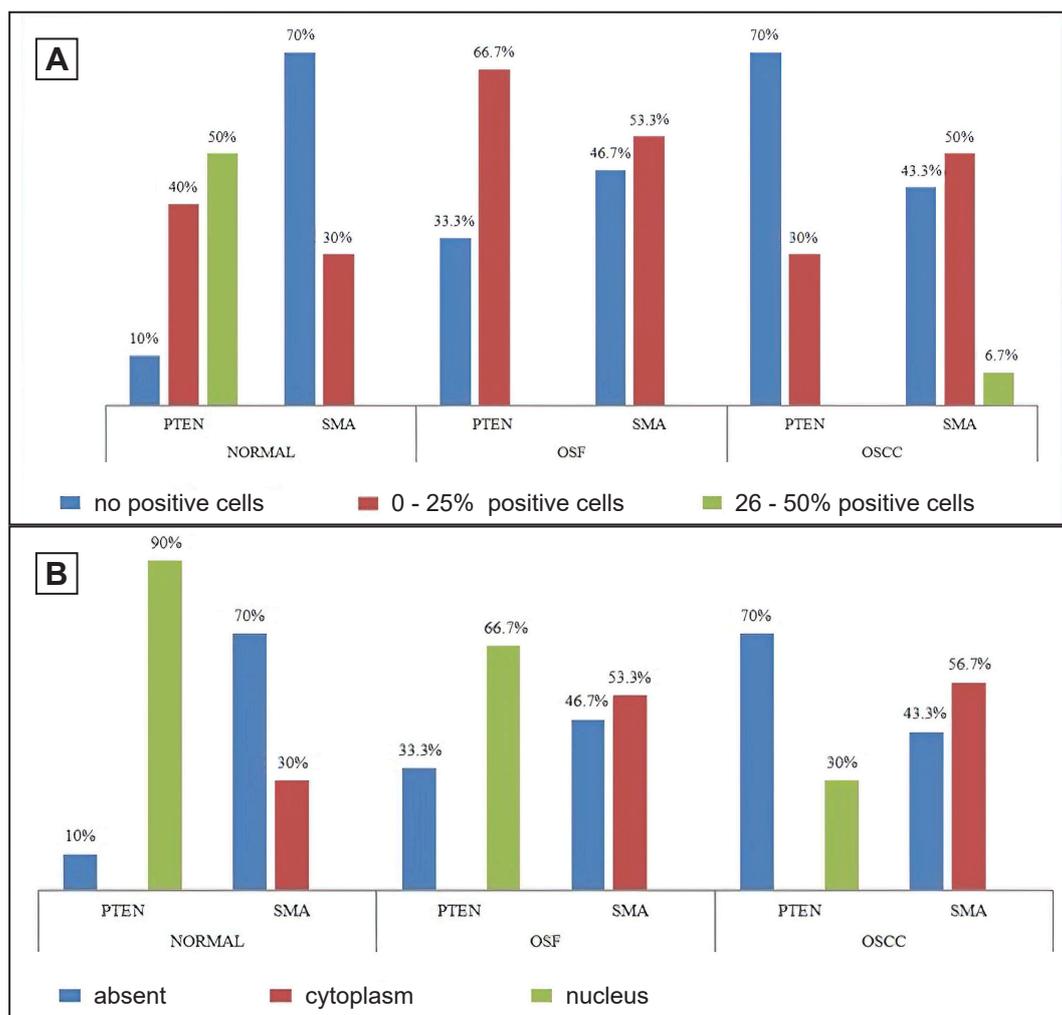

Figure 5. A = comparison of percentage positivity of phosphatase and tensin homologue and alpha-smooth muscle actin.
B = comparison of distribution of pattern of phosphatase and tensin homologue and alpha-smooth muscle actin.

DISCUSSION

PTEN, on chromosome 10q23 is "the most highly mutated gene in the post p53 era", encoding a protein which dephosphorylates both protein and lipid substrates and negatively regulates the PI3K/AKT pathway [21-23]. Normally, the lipid phosphatase dephosphorylates PIP3, opposing PI3K and inhibits activation of the downstream pathway [24]. The dephosphorylation inactivates substrates making it a tumour suppressor gene. PTEN inactivation leads to PI3K/AKT activation and phosphorylation of PIP2 in the 3' position of the inositol ring at the cell membrane leading to PIP3 synthesis and initiation of G1 phase of cell cycle and reduction in apoptosis, thereby predisposing to the development of cancer [21,22]. PTEN has been studied in precancers of endometrium, leukoplakia; cancers of breast, prostate, endometrium and head and neck [9,21,22,25]. PTEN also negatively regulates cell survival and fibroblast proliferation in the pathogenesis of fibrotic disorders like OSF, lung fibrosis, dermal fibrosis, hepatic fibrosis [6,14,15,21].

The clinicopathological parameters in relation to OSF in the present study were in accordance with that of previous studies where predominantly males in the second and third decades of life with the habit of chewing ghutka and other commercially available areca nut preparations for more than 10 years were affected by OSF; with the severity related to frequency and duration of habit [26,27]. OSCC concomitant with OSF, in contrast to our study has been reported to affect males below 50 years of age while OSCC without OSF in patients above 50 years of age, probably because patients suffering from OSCC without concomitant OSF do not normally experience pain and discomfort till the lesion progresses to a functionally restrictive stage [28-30]. Likewise, males with the habit of consuming commercially available areca nut preparations for more than 5 years have been reported to be at higher risk of OSCC with OSF [29,30]. In OSCC with OSF, the fibrous stroma limiting tumour infiltration, accounts for better prognosis than in OSCC without OSF by means of tumour depth and differentiation. Also lymph node metastasis and extracapsular spread (ECS) is less in OSCC arising from OSF [29,30]. However, controversy still exists regarding the prognosis of OSCC with and without OSF.

Apart from the clinicopathological aspect, oral carcinogenesis encompasses mutations and epigenetic abnormalities in the activation of oncogenes and inactivation of tumour suppressor genes that control cell growth, inhibit cell proliferation by inducing apoptosis and tumour development [31]. With this background, we undertook this study to know more about the molecular changes in the malignant transformation of OSF. Tumour suppressor genes p53, p16, retinoblastoma (RB), PTEN have been studied in OSF to assess their role in progression of cancer and prediction of malignant transformation [32,33]. Likewise, we studied the expression of PTEN in normal mucosa, OSF and OSCC with concomitant OSF.

An intense nuclear PTEN staining seen in basal and parabasal layers in nearly all normal and OSF epithelium pointed out its normal function, but the percentage of positivity gradually reduced in OSF group, probably indicating the 33.3% OSF cases with loss of expression are more prone to malignant transformation. This was substantiated by the OSCC group exhibiting a further gradual decrease in the number of positive cells (i.e., 70% were negative), seen at the periphery of tumour islands. Angadi and Krishnapillai [21], have also reported an intense nuclear stain in basal and parabasal layers of normal and OSF epithelium that reduced progressively from OSF to OSCC with positive cells at the tumour island periphery. Nuclear PTEN maintains chromosomal stability through physical interaction with centromeres and control of DNA repair [34]. Contrastingly, Jasphin et al. [35] on assessing the expression of PTEN between grades of OSCC, noted positivity in the cell cytoplasm; predominantly in basal and spinous layers with intensity reducing towards the granular layer. However, in spite of a decrease in the cells staining for PTEN in OSCC compared to the normal tissues, no statistical significance was seen in expression among the grades.

While nuclear PTEN is more stable and retains the capability of inhibiting AKT and inducing cell death, it downregulates cyclin D1 and phospho-MAPK and is thus crucial for cell cycle arrest [36,37]. Compared to cytoplasmic staining, nuclear staining of PTEN is more marked particularly in undifferentiated and metastatic tumours of thyroid and melanoma. But the role of this shift in cellular localization in tumour pathogenesis is yet to be understood [38].

An increase in PTEN expression predominantly in basal and parabasal cell cytoplasm in dysplasia may be considered an attempt to control the increased AKT expression. In leukoplakia, PTEN allelic loss is an important mechanism causing progression to oral cancer [25].

The expression and genetic alterations of PTEN in OSCC have shown the epigenetic changes to be related to a down regulation of the protein [39].

The homozygous deletion or point mutation may not play a crucial role in PTEN inactivation process of carcinomas of oral cavity [40]. Activation of AKT is known to be associated with advanced stages and poor prognosis in OSCC; and induced PTEN over-expression downregulates AKT phosphorylation and encourages apoptosis [35].

In malignancies of human brain, breast and prostate, PTEN mutations have been assessed [8]. Squarize et al. [24], found multiple HNSCC lesions in PTEN deficient mice. Mutter et al. [9], reported PTEN mutations in 83% of endometrial cancers and 55% of precancers; most had mutation in only one PTEN allele. Roa et al. [22], noted that patients with reduced PTEN expression died within 10 months indicating poor prognosis.

Functionally, PTEN controls/regulates p53 by preventing its degradation through PI3K signalling pathway [41,42]. Inactivation of PI3K pathway, leads to decreased phosphorylation of mouse double minute 2 homolog (MDM2) by AKT and translocation of MDM2 to cytoplasm. Cytoplasmic MDM2 cannot ubiquitinate nuclear p53 and so p53 levels increase. Thus p53 increases PTEN levels and, conversely, PTEN increases p53 levels [8,43]. Studies in mammary tumours and liposarcomas showed that p53 combined with PTEN might be an essential blockage in their development [44,45].

In consort with tumour suppressor genes, the stroma comprising inflammatory cells, small vessels, fibroblasts, myofibroblasts, and ECM components is vital in controlling tumour growth and differentiation. Myofibroblasts, the key cellular mediators of fibrosis in OSF express α-SMA, produce collagen, ECM proteins and proteolytic enzymes causing matrix degradation and tumour invasion [12,46,47]. PTEN playing a role in embryogenesis, immunity, fibrosis and malignancy is known to have an inverse correlation with α-SMA [11,48,49]. Thus PTEN acts both as a tumour suppressor and controller of myofibroblast.

PTEN gene inactivation results in TGF β1 induced disassembly of tight junction of epithelial cells, destruction of basement membrane and increased epithelial derived myofibroblast due to up-regulation of AKT/S6K/Snail pathway, playing a role in epithelial-mesenchymal transition (EMT) [14].

According to Xie et al. [50], PTEN overexpression decreases signalling via the PI3K/AKT and TGF β/Smad3 pathways in IPF and inhibits TGF β1 mediated myofibroblast differentiation. An increased TGF β in OSF can reduce PTEN levels allowing for AKT activity and consequently prolonged survival of fibroblasts and increased ECM production

and fibrosis [21]. Deletion of PTEN in mouse fibroblasts raises AKT phosphorylation and expression of connective tissue growth factor (CTGF/CCN2) and an escalation in dermal thickness owing to excess collagen deposition. An elevated selective inhibition of PI3K/AKT pathway reduces overexpression of collagen and CCN2. Thus, PTEN appears to be a potential regulator of fibrogenesis [6].

In the present study, an intense α-SMA staining in myofibroblast cytoplasm, relatively more in OSF and OSCC than in normal mucosa with gradual increase both from normal to OSF and OSF to OSCC was witnessed. But a statistical significance difference failed to exist. In OSF group, 12 cases were in the combined stage I and II, 4 were in the combined stage III and IV. Similar observations with an increase in number of α-SMA stained myofibroblasts in OSF compared to normal mucosa, but with a statistically significant increase in population between early and advanced stages have been reported, demonstrating that myofibroblasts could serve as effective prognostic markers for disease progression in OSF [12,13,51,52]. Alka et al. [51], revealed high α-SMA expression in OSCC with and without OSF compared to OSF, which indicated despite the statistically insignificant difference, altered ECM in OSCC with OSF may perhaps be responsible for modified EMT. In OSCC not associated with OSF, expression of α-SMA is greater in OSCC than in OSF alone or OSF associated with dysplasia. The proteolytic enzymes secreted by activated myofibroblasts cause matrix degradation, contributing to cancer cell invasion and metastasis [12,16]. In OSCC arising from OSF, EMT occurs first when areca nut induced cell injury causes excessive collagen cross-linking in ECM; second when there is malignant transformation during which cytokines and growth factors released from cancer cells induce myofibroblast transdifferentiation [53].

A positive correlation between OSCC histological grades and myofibroblasts has not been established, suggesting formation and differentiation of myofibroblasts is induced somewhere in the invasive stage of OSCC, and an increase in severity and differentiation would not affect their number [54,55]. Also variation in TGF β secretion essential for myofibroblast differentiation by tumour cells could be a reason for the decrease in myofibroblasts [51].

We noted a gradual decrease in PTEN expression and a gradual increase in α-SMA expression from normal to OSF and OSCC, providing a weak inverse correlation between PTEN and α-SMA. In IPF, an inverse correlation between PTEN and α-SMA expression has been established and α-SMA is the hallmark of mature myofibroblasts [56,57].

Once fibrosis is established, there can be either persistence without significant progression of disease or eventual malignant transformation [52]. An increase in number of myofibroblast both in OSCC associated and not associated with OSF indicates a poor prognosis [19,21]. Thus PTEN controlling fibroblast differentiation into myofibroblast plays a definite role in carcinogenesis. However, the present study suffered from certain shortcomings. A larger sample size and a follow-up of the cases would certainly aid in knowing the response to the treatment and a better validation of the results.

CONCLUSIONS

The presented study concludes the absence of phosphatase and tensin homologue positivity in oral submucous fibrosis suggests these cases have a greater tendency to progress towards malignancy. Thus phosphatase and tensin homologue plays a crucial role in tumorigenesis of oral squamous cell carcinoma. In addition, alpha-smooth muscle actin has an effective prognostic role in disease progression of oral submucous fibrosis.

The weak inverse correlation between phosphatase and tensin homologue and alpha-smooth muscle actin showed that phosphatase and tensin homologue aids in myofibroblast differentiation in oral submucous fibrosis, further increasing fibrosis and desmoplasia.

Thus alteration of phosphatase and tensin homologue and alpha-smooth muscle actin is likely an important molecular event in pathogenesis of oral submucous fibrosis and its malignant transformation. Since phosphatase and tensin homologue is involved in the PI3K/AKT pathway, drugs targeting PI3K itself might have significant therapeutic activity in phosphatase and tensin homologue null cancers.

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