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EXPRESSION OF SMAD-4 IN ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA: A COMPARATIVE STUDY

Dr. Diviani Feria[1], Dr. Gunveen Kaur[2], Dr. Mandeep Kaur[3]

[1]Senior Lecturer, Swami Devi Dayal Dental College, Golpura (Barwala).
Senior Lecturer, Institute of Dental Sciences, Sehora, Jammu
Assistant Professor, Dept of Oral Pathology & Microbiology, Indira Gandhi Govt Dental College, Jammu.

Corresponding Author: Dr. Gunveen Kaur
[1]Senior Lecturer, Institute of Dental Sciences, Sehora
Email: kaurgunveen9@gmail.com

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ABSTRACT:

Introduction: The World Health Organization (WHO)points out the following lesions as the main oral potentially malignant disorders: leukoplakia, erythroplakia, actinic cheilitis, oral submucous fibrosis and lichen planus. Oral Cancer accounts for approximately 3% of all malignancies and found in 270,000 patients annually worldwide.

Aims: To study the expression of SMAD-4 in oral potentially malignant disorders and oral squamous cell carcinoma and its role as a prognostic marker.

Materials & Methods: 20 specimens of OPMD, 20 OSCC and10 of normal mucosa were taken. They were stained with standard H&E and IHC using primary antibody SMAD 1. Expression of SMAD-4 protein was determined by staining quantitative assessment of the percentage of marked tumor cells. Group comparisons were made with the Chi-Sq test as data was skewed so comparisons for two groups were made by Mann-Whitney test. Correlations between scores were calculated with Spearman correlation coefficient. All statistical tests were two-sided and performed at a significance level of α=.05. Analysis was conducted using IBM SPSS STATISTICS (version 22.0).

Results: On comparing the staining intensities of group 1 (OPMD) and group 2 (OSCC) statistically highly significant difference is obtained (p value=.001) and on comparison of percentages of immunopositive cells of group 1 (OPMD) and group 2 (OSCC) statistically highly significant difference is obtained (p value=.029).

Conclusion: SMAD-4 was a tumor suppressor and loss of SMAD-4 expression may lead to spontaneous oral squamous cell carcinoma development, patients whose oral potentially malignant lesions with higher levels of SMAD-expression displayed a significantly higher rate of malignant transformation.

Keywords: Oral premalignant disorders, Oral squamous cell carcinoma, SMAD -4

1. INTRODUCTION

In 1978, World Health Organization (WHO) proposed the terms "precancerous conditions" and "precancerous lesions" and defined precancerous lesion as "a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart." Oral potentially malignant disorders are usually found on the buccal mucosa, followed by gingivae, tongue and floor of the mouth. Prevention and early detection of oral potentially malignant disorders have the potential of not only decreasing the incidence, but also in improving the survival of the person with the preexisting disorder. Among the varied spectrum of oral potentially malignant disorders, oral leukoplakia is known to be the most common one. Oral leukoplakia is associated with a 40.8-fold increased risk of oral cancer and a 5-year absolute risk of 3.3% (1 in 30 individuals progressing to cancer over 5 years)[1]. SMAD proteins are transcriptional regulators activated by TGF-β. They are known to bind to two distinct SMAD responsive motifs, namely the SMAD-binding element (SBE) (5′-GTCTAGAC-3′) and CAGA motifs (5′-AGCCAGACA-3′ or 5′- TGTCTGGCT-3′). The role of SMAD-4 as a tumor suppressor was initially identified in pancreatic cancer-4 or DPC-4 (deleted in pancreatic cancer-4). 10 Loss expression of SMAD-4 was associated with poor clinical outcomes in patients with pancreatic, colon and brain cancers[2]. Main aim of this study was to evaluate the expression and distribution patterns of SMAD-4 in oral potentially malignant disorders and oral squamous cell carcinoma.

2. MATERIALS & METHODS

This was a laboratory based Retrospective and Analytical study. Duration of this study was approximately 7-10 months.

Inclusion criteria

a. Blocks of Histologically proved cases of Oral Potentially Malignant Disorders and oral squamous cell carcinoma.
b. Adults.

Exclusion criteria

a. Cases showing evidence of malignancy / micro invasion.
b. Cases which cannot be diagnosed histologically
c. Cases where epithelium is not seen histologically.
d. Slides with tissues which are not representative of the pathology.

Paraffin embedded tissue specimens of diagnosed cases of Oral Potentially Malignant Disorders and Oral squamous cell carcinoma were retrieved from the archives of the Department of oral Pathology and oral Microbiology of Swami Devi Dyal Dental Hospital and College, Barwala.
The study specimens included were categorized as follows:
GROUP 1: 20 specimens Oral potentially Malignant Disorders
GROUP 2: 20 specimens of Oral Squamous Cell Carcinoma
CONTROL: 10 normal oral mucosa biopsies from gingivectomy cases, impaction cases, stage 3 implant cases.

Data on patient age, gender and lesion site were be obtained from the biopsy requisition forms submitted. The tissue sections thus obtained were stained using the following methods:
1. Standard Hematoxylin and Eosin (H&E) Stain.

2. Immunohistochemical Staining Using Primary Antibody SMAD-4 Stain.

Immunostaining Evaluation:
The evaluation of staining was done by the following method used by Sakata J et al in their study. Staining Quantity score:
Expression of SMAD-4 protein was determined by staining quantitative assessment of the percentage of marked tumor cells as shown in following table. For each specimen, one score was assigned according to the percentage of positive cells.
Less than 5%- 1 point
6-35% - 2 point
36-70% - 3point
More than 70%- 4 point

Staining Intensity score:
Assessment of staining intensity reaction was considered using a second score assigned according to the intensity of the staining, with negative staining equaling 1 point, weak staining equaling 2 points, moderate staining equaling 3 points, and strong staining equaling 4 points.

The SMAD-4 labeling index: It is defined as the weighted percentage of epithelium cells displaying nuclear staining multiplied by the degree of the staining intensity.
For each specimen, one score was assigned according to the percentage of positive cells: Less than 5%- 1 point, 6-35% - 2 point, 6-70% - 3point, 71%- 4 point. A second score was assigned according to the intensity of the staining, with negative staining equaling 1 point, weak staining equaling 2 points, moderate staining equaling 3 points, and strong staining equaling 4 points. SMAD-4 expression scores were then calculated by multiplying the two scores described above.
Immunoreactive Score (IRS): Scores for the percentage of immune positive tumor cells and scores for the staining intensities will be multiplied to calculate the immunoreactive score (IRS) as shown in the following table (A× B= IRS).

Immunoreactive Score (IRS): Scores for the percentage of immune positive tumor cells and scores for the staining intensities will be multiplied to calculate the immunoreactive score (IRS) as shown in the following table (A× B= IRS).
If the expression score was <4, the tissue was considered as low expression and if expression score is ≥4, the tissue was considered as high expression.

A(Percentageofpositive cells)	B(Intensityofstaining)	IRS(Multiplicationof A× B)
0=No positive cells	1=Negativestaining	0-1=negative
1=<5%positive cells	2=Weak staining	2-3=mild
2=6-35%positivecells	3=Moderatestaining	4-8=Moderate
3=36-70%positivecells	4=Strong staining	9-12= stronglypositive

4=>70%positivecells	Final Immunoreactive score(A×B):0-12

Statistical Analysis:
Normality of quantitative data were checked by measures of Kolmogorov Smirnov tests of Normality. Our data was skewed so data were given as mean ± SD, range and median with interquartile range.Age was normally distributed so it was compared with t-test and was presented as mean, SD with range. Gender was reported as counts and percentages. Group comparisons were made with the Chi-Sq test as data was skewed so comparisons for two groups were made by Mann-Whitney test. Correlations between scores were calculated with Spearman correlation coefficient. All statistical tests were two-sided and performed at a significance level of α=.05. Analysis was conducted using IBM SPSS STATISTICS (version 22.0).

3. RESULTS

SAMPLE DISTRIBUTION

Table 1: Table Showing Sample Distribution

GROUPS	Total
GROUP 1: (STUDY GROUP) ORAL POTENTIALLY MALIGNANT DISORDERS	20
GROUP 2: (STUDY GROUP) ORAL SQUAMOUS CELL CARCINOM	20
GROUP 3: (CONTROL) NORMAL MUCOSA	10
Total	50

Table 1 shows sample distribution. Group1 comprises histologicallydiagnosed 20 cases of oral potentiallymalignant disorders (n= 20). And Group 2 comprises of histologically diagnosed 20 cases of oral squamous cell carcinoma (n= 20). 10 cases of normal oral mucosa were taken as control (n=10).

Table 2: Showing Age Distribution Within The Study Group

STUDY GROUP	NUMBER OF CASES	MEAN AGE	SD
GROUP 1 (ORAL POTENTIALLY MALIGNANT DISORDERS)	20	35.30	±11.407
GROUP 2 (ORAL SQUAMOUS CELL CARCINOMA	20	48.00	±12.929
TOTAL	40	41.65	±13.64

Table 2 shows that mean age of Group 1 (Oral potentially malignant disorders) is 35.30±11.407 whereas mean age of Group 2 (oral squamous cell carcinoma) is 48±12.929.

Table3: Showing Gender Distribution In Study Group.

GENDER		STUDY GROUP		
		GROUP 1	GROUP 2	TOTAL
FEMALE	Count	6	7	13
	%within group	30%	35%	32.50%
MALE	Count	14	13	27
	%within group	70%	65%	67.50%
TOTAL	Count	20	20	40
	%within group	100%	100%	100%

Table 3 shows that males in total sample are 27 (67.5%) and females in total sample are 13 (32.5%) out of 40. There are six females (30%) and fourteen males (70%) in Group 1(Oral Potentially Malignant Disorders) whereas there are seven females (35%) and thirteen males (65%) in Group 2 (oral squamous cell carcinoma).

Table 4: Showing Staining Intensity Score In Group 1 (Oral Potentially Malignant Disorders) After Immunohistochemical Staining With Smad-4

STAINING INTENSITY SCORE		NO. OF CASES (n=20)
Score 1 (negative staining)	Count	02
	%within group	10%
Score 2 (weak staining)	Count	08
	%within group	40%
Score 3 (moderate staining)	Count	04
	%within group	20%
Score 4 (strong staining)	Count	06
	%within group	30%
TOTAL	Count	20
	%within group	100.0%

Table 4 shows that in Group 1(Oral potentially malignant disorders) there are six slides (30%) out of 20 showing strong staining with SMAD-4 (score 4), four slides (20%) out of 20 showing moderate staining with SMAD-4 (score 3), eight slides (40%) out of 20 showing weak staining with SMAD-4 (score 2) and two slides (10%) out of 20 showing negative staining with SMAD-4 (score 1).

Table 5: Showing Staining Intensity Score In Group 2 (Oral Squamous Cell Carcinoma) After Immunohistochemical Staining With Smad-4.

STAINING INTENSITY SCORE		**NO. OF CASES (n=20)**
Score 1 (negative staining)	Count	17
	%within group	85%
Score 2 (weak staining)	Count	03
	%within group	15%
Score 3 (moderate staining)	Count	00
	%within group	00%
Score 4 (strong staining)	Count	00
	%within group	0.0%
TOTAL	Count	20
	%within group	100.0%

Table 5 shows that in Group 2 (oral squamous cell carcinoma) there are three slides (15%) out of 20 showing weak staining with SMAD-4 (score 2), seventeen slides (85%) out of 20 showing negative staining with SMAD-4 (score 1).

Table 6: Showing Comparison Of Staining Intensity Score Between Group 1 (Oral Potentially Malignant Disorders) And Group 2 (Oral Squamous Cell Carcinoma).

SMAD-4* GROUP CROSS TABULATION				
STAINING INTENSITY SCORE		**STUDY GROUPS** **GROUP 1**	**GROUP 2**	
Score 1 (negative staining)	Count	02	17	19
	%within group	10%	85%	47.5%
Score 2 (weak staining)	Count	08	03	11
	%within group	40%	15%	27.5%
Score 3 (moderate staining)	Count	04	00	04
	%within group	20%	00%	10%
Score 4 (strong staining)	Count	06	00	06
	%within group	30%	00%	15%
TOTAL	Count	20	20	40
	%within group	100.0%	100.0%	100.0%

Table 6 shows that on comparison of staining intensity scores of Group 1 (Oral potentially malignant disorders) and Group 2 (oral squamous cell carcinoma) the maximum cases of group 1 exhibited weak staining with SMAD-4 (score 2) whereas majority of cases of group 2 exhibited negative staining with SMAD-4 (score 1).

Statistical Analysis:

Study Group	P value	Significance
Group1 (OPMD)	.001	Significant
Group2 (OSCC)		

Correlation between staining intensity scores of Group 1 and Group 2 is highly significant. (p value=.001)

Table 7: Showing Staining Quantity Score In Group 1 (Oral Potentially Malignant Disorders) After Immunohistochemical Staining With Smad=4.

STAINING QUANTITY SCORE		NO.OF CASES (n)
Score 0 (no positive cells)	Count	02
	%within group	10.0%
Score 1 (<5% of positive cells)	Count	08
	%within group	40%
Score 2 (6-35% positive cells)	Count	05
	%within group	25%
Score 3 (36-70% positive cells)	Count	04
	%within group	20%
Score 4 (>70% positive cells)	Count	01
	%within group	05%
Total Count		20
TOTAL% within group		100.0%

Table 7 shows that in Group 1 (Oral potentially malignant disorders) one out of 20 slides (05%) have >70% positive cells (score 4), four out of 20 slides (20%) have36-70% positive cells (score3), five out of 20 slides (25%) have 6-35% positive cells (score 2), eight out of 20 slides (40%) have <5% (score 1) and two out of 20 slides (10%) have no positive cells (score 0) towards SMAD-4.

Table 8: Showing Staining Quantity Score In Group 2 (Oral Squamous Cell Carcinoma) After Immunohistochemical Staining With Smad-4.

STAINING QUANTITY SCORE	NO. OF CASES (n)

Score 0 (no positive cells)	Count	15
	%within group	75%
Score 1 (<5% of positive cells)	Count	03
	%within group	15%
Score 2 (6-35% positive cells)	Count	02
	%within group	10%
Score 3 (36-70% positive cells)	Count	00
	%within group	0.0%
Score 4 (>70% positive cells)	Count	00
	%within group	0.0%
TOTAL	Count	20
	%within group	100.0%

Table 8 shows that in Group 2 (oral squamous cell carcinoma) two out of 20 slides (10%) have 6-35 % positive cells (score 2), three out of 20 slides (15%) have <5% positive cells (score 1) and fifteen out of 20 slides (75%) have no positive cells (score 0) towards SMAD-4.

Table 9: Showing Comparison of Staining Quantity Score between Group 1 (Oral Potentially Malignant Disorders) and Group 2 (Oral Squamous Cell Carcinoma)

GROUP CROSS TABULATION				
STAINING QUANTITY SCORE		**STUDY GROUPS GROUP 1 GROUP 2**		**TOTAL**
Score 0 (no positive cells)	Count	02	15	17
	%within group	10%	75%	42.5%
Score 1 (<5% positive cells)	Count	08	03	11
	%within group	40%	15%	27.5%
Score 2 (6-35% positive cells)	Count	05	02	07
	%within group	25.0%	10%	17.5%
Score 3 (36-70 %positive cells)	Count	04	00	04
	%within group	20.0%	0.0%	10%
Score 4	Count	01	00	01

(>70% positive cells)	%within group	05.0%	0.0%	2.5%
Total	Count	20	20	40
	%within group	100.0%	100.0%	100.0%

Table 9 shows that on comparison of staining quantity score of Group 1(Oral potentially malignant disorders) and Group 2(oral squamous cell carcinoma) the maximum cases of Group 1(Oral potentially malignant disorders) exhibited <5% of positive cells (score 1) where as majority of cases of Group 2 (oral squamous cell carcinoma) exhibited no positive cells (score 0) towards SMAD-4.

Statistical Analysis:

Study Group	P value	Significance
Group1 (OPMD)	.029	Significant
Group2(OSCC)		

CorrelationbetweenstainingquantityscoresofGroup1andGroup2issignificant. (p value=.029)

Table 10: Showing Immunoreactive Score (Irs) Of Group 1 (Oralpotentially Malignant Disorders)

IMMUNOREACTIVESCORE (IRS)	NUMBER OF CASES (n)
NEGATIVE (0-1)	02
MILD (2-3)	07
MODERATE (4-8)	08
STRONGLY POSITIVE (9-12)	03
TOTAL	20

Table 10 shows that in Group 1 (Oral potentially malignant disorders) two slides out of 20 (10%) exhibit negative expression, seven slides out of 20 (35%) exhibit mild expression, eight slides out of 20 (40%) exhibit moderate expression, three slides out of 20 (15%) exhibit strong expression towards SMAD-4.

Table 11: Showing Immunoreactive Score (Irs) Of Group 2 (Oral Squamous Cell Carcinoma)

IMMUNOREACTIVE SCORE(IRS)	NUMBER OF CASES (n)
NEGATIVE (0-1)	16
MILD (2-3)	04
MODERATE (4-8)	00

STRONGLY POSITIVE (9-12)	00
TOTAL	20

Table 11 shows that in Group 2 (oral squamous cell carcinoma) four slides out of 20 (20%) exhibit mild expression and sixteen slides outof 20 (80%) exhibit negative expression towards SMAD-4.

Table 12: Showing Comparison Of Immuno Reactive Score (Irs) Between Group 1 (Oral Submucous Fibrosis) And Group 2 (Well Differentiated Squamous Cell Carcinoma).

GROUP CROSS TABULATION				
IRS SCORE		**STUDY GROUPS**		**TOTAL**
		GROUP 1	**GROUP 2**	
0-1	Count	02	16	18
	%within Group	10%	80.0%	45%
2-3	Count	07	04	11
	%within Group	35%	20.0%	27.5%
4-8	Count	08	00	08
	%within Group	40.0%	0.0%	20%
9-12	Count	03	00	03
	%within Group	15%	00%	7.5%
Total	Count	20	20	40
	%within Group	100.0%	100.0%	100.0%

Table 12 shows that on comparison of Immunoreactive score (IRS) of Group 1 (Oral potentially malignant disorders) and Group 2 (oral squamous cell carcinoma) the maximum cases of Group 1 (Oral potentiallymalignant disorders) exhibited moderate expression (score 3) whereas majority of cases of Group 2 (oral squamous cell carcinoma) exhibited strongly negative expression (score 0) towards SMAD-4.

Statistical Analysis:

Study Group	P value	Significance
Group1 (OPMDS)	<.001	Significant
Group2 (OSCC)		

Correlation between immuno reactive scores of Group 1and Group 2 is highly significant. (p value=<.001)

Table 13: Showing Overall Mean And Sd Of Staining Intensity, Staining Quantity And Immunoreactive Score (Irs) Of Both Group 1 (Oral Potentially Malignant Disorders) And Group 2 (Oral Squamous Cell Carcinoma).

STUDYGROUP		N	MEAN	SD
GROUP 1 (ORALPOTENTIALLY	Staining intensity score	20	2.70	1.031

MALIGNANT DISORDERS)	Staining quantity score	20	1.70	1.081
	Immunoreactive score	20	4.55	3.748
GROUP 2 (ORAL SQUAMOUS CELL CARCINOMA)	Staining intensity score	20	1.15	0.366
	Staining quantity score	20	0.35	0.671
	Immunoreactive score	20	1.20	0.768

Table 13 shows that on comparison the overall mean of Group 1 (Oral potentially malignant disorders) staining intensity score is 2.7, staining quantity score is 1.7, immunoreactive score is 4.55 and whereas the overall mean of Group 2 (Oral squamous cell carcinoma) staining intensity score is 1.15, staining quantity score is 0.35, immunoreactive score is 1.2

4. Discussion

Head and neck cancers are aftermath of diverse hetrogeneous abnormalities and intricate molecular irregularities which accounts for significant morbidity and mortalitiy. OSCC has emerged as a prime carcinoma among copious other head and neck cancers, accounting for 3% of all malignancies and evolving as one of the most common malignant tumor. Oral squamous cell carcinoma (OSCC), which comprises approximately half of head and neck cancer, is the most common subtype of head and neck carcinoma.[3] The 5-year survival rate of patients with OSCC remains almost unchanged despite various treatment improvements in the last three decades.[4]

Many potentially malignant disorders such as leukoplakia, erythroplakia and oral submucous fibrosis behold as one of the initiation factor for OSCC. Oral leukoplakia (OL) is the most common premalignancy in the oral cavity and can progress to oral squamous cell carcinoma(OSCC).[5]TGF-β signaling pathway plays an important role in embryonic development and in the regulation of tissue homeostasis.[6] Previous reports showed that TGF-β possessed dual functions: it functioned as a tumor suppressor in the initiation steps of tumorigenesis by inhibiting proliferation and inducing apoptosis while in the later stages of tumorigenesis and progression by inducting epithelium-mesenchymal transition (EMT), stimulating angiogenesis and suppressing immune system.[5] SMAD-4 functions as common SMAD (CO-SMAD)to mediate TGF-beta and BMP signaling pathway In HNSCC, loss of heterozygosity at SMAD-4 gene region was observed in 30%–50%ofthe tumors, suggesting a tumor suppressor role of SMAD-4.[5,7]

In the present study, study group was categorised into two Groups namely Group 1 which comprised of 20 cases of histologically diagnosed cases of Oral potentially malignant disorders and Group 2 Oral squamous cell carcinoma. 10 cases of normal oral mucosa were taken as control.

In the present study mean age of study sample was observed to be 41.65±13.64. Further, in our study, mean age of patients of oral potentially malignant disorders is 35.30±11.407 years and oral squamous cell carcinoma is 48±12.92 years (Table 2), which was in accordance to

the study done by Molook Torabietal[45] .In their study
the mean age of patients in OPMDS was 46.82 ± 15.22 years but in OSCC with the mean patient age of 59.44 ± 17.55 years which is slightly high than our study. In another study by Shenoi R et al mean age of patient of Oral squamous cell carcinoma is 49.3 years which is in accordance with the present study.[9]
In the present study we observed male predominance with 67.5% males and female: male ratio turns out to be 1:2.1. Group 1 (Oral potentially malignant disorders) comprised of 70% males and 30% females while Group 2 (Oral squamous cell carcinoma) comprised of 65% males and 35% females (Table 3). In similarity to the present study Kumar S et al also reported male predominance with 59.2% males.[10] In a review done by Nair et al., the prevalence of oral potentially malignant disorders and oral cancer was found to be more in males.[11] A similar finding was reported in study conducted by Lin et al. in Taiwan, wherein a statistical significant difference was observed between various oral potentially malignant disorders detected and gender.[12] A reason that the authors believe for this gender discrepancy with males being at higher risk may be due to the fact that the habit of tobacco consumption is more in males which may lead to development of oral potentially malignant disorders in males.
Negative expression of SMAD-4 is seen in normal oral mucosa (n=10) indicating it as a normal control. In accordance with the present study Bornstein et al reported similar findings.[13] In their study they initially determined that the cancers in patients with HNSCC expressed decreased levels of SMAD-4 in normal buccal mucosa which implied that SMAD-4 loss occurs early during the development of HNSCC in humans.[13]

IMMUNOHISTOCHEMICAL EVALUATION OF EXPRESSION OF SMAD-4 IN ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA.

Assessment of Staining intensity:

In Group 1 (Oral Potentially Malignant Disorders) 30% cases showed strong staining with SMAD-4, 20% showed moderate staining with SMAD-4, 40% showed weak staining with SMAD-4and in10%SMAD-4 was negatively expressed (Table4).
Thus, from our results we interpret that there was a mixed pattern of SMAD-4 with 40% of cases exhibiting weak SMAD-4 expression and 30% cases exhibited strong SMAD-4 expression. These findings are in accordance with the previous studies done by Sakata J et al[14] and Xia RH et al.[5] In their studies, Xia RH et al reported 48.9% cases with strong SMAD-4 expression and 51.1% cases with weak SMAD -4 expression[14] while Sakata J et al reported 56% of cases with strong SMAD-4 expression and 44% cases showed weak SMAD-4 expression. These authors concluded that SMAD-4 appears to play a role in malignant transformation of OMPDS. Further, they also reported an association between a higher SMAD-4 expression and an increased rate of OL malignant transformation.
In Group 2 (Oral squamous cell carcinoma) there are three 85% of cases showed negative staining with SMAD-4 whereas 15% cases showed weak staining with SMAD-4. Hence, from our results we can interpret that a predominant low SMAD-4 expression was exhibited by the OSCC samples. These results can be justified by a study done Bornstein et al according to them 86% of HNSCC samples exhibited down regulation of SMAD-4.[13]The lower SMAD-4 expression in OSCC tissues was also in accordance with the other studies which showed that SMAD-4 was a tumor suppressor in OSCC.[5]
On comparison, a statistically significant difference is observed on comparison of staining intensities of both the groups (p=.001) (Table 6). Similar results of SMAD-4 staining intensity were observed by Sakata J et al[14] and Xia RH et al[5]. Xia RH et al in his study concluded that SMAD-4 loss can be significantly correlated with the malignant

transformation of oral leukoplakia and can be used for the prognosis of OSCC patients.[5]

Assessment of Staining quantity:

In the present study quantitative expression of SMAD-4 or the percentage of immunopositive tumor cells in Group 1 (Oral potentially malignant disorders) shows that 5% cases have >70% positive cells, 20% cases have 36-70% positive cells, 25% have 6-35% positivecells,40% cases have<5% positive cells and10% have no positive cells. Thus, predominantly >5% of immunopositive tumor cells were seen in Oral potentially malignant disorders in our study with 40% cases followed by 6-35 % of immunopositive tumor cells in 25% cases.

In Group 2 (Oral squamous cell carcinoma) 10% cases have 6-35% positive cells,15% have >5% positive cells and 75% have no positive cells (Table 8). Hence, predominantly >5% of immunopositive tumor cells were seen in OSCC specimens in the present study.

On comparison, a statistically significant difference is observed between staining quantities of both the Groups (p value=.029) (Table 9). These findings were in accordance with the previous studies conducted by Xia RH et al.[5]

Assessment of Immunoreactive scoring

Low expression of SMAD-4 is defined based on a combination of the percentage of stained cells and the intensity of staining. Thus, scores of the percentage of immunopositive cells and cellular expression intensity is multiplied to calculate an immunoreactive score (IRS), this method previously described by Remmeleand Stegner.[15]

In our study, in Group 1 (Oral potentially malignant disorders) 10% cases exhibited negative expression, 35% exhibited mild expression, 40% exhibited moderate expression and 15% of cases exhibited strongly positive expression towards SMAD-4 (Table 10). Hence, overall predominantly moderate SMAD-4 expression was seen in OPMDS in our study with 40% of the cases.

In Group2 (Oral squamous cell carcinoma) 80% exhibit negative expression and 20% exhibit mild expression towards SMAD-4 (Table 11). Hence, overall predominantly negative SMAD-4 expression was exhibited by OSCC specimens in our study.

On comparing the immunoreactive scores of both the groups: Group 1 and Group 2 (OPMDS AND OSCC) a statistically significant difference is observed. (p value=.034). (Table12). These findings were in concordance with study conducted by Xia RH et al.[5]

Thus, in the present study, statistically significant over all staining intensity (p=.003), percentage of immunopositive cells (p=.001) and immunoreactive scoring (p=<.001) was obtained by SMAD-4 in Group 1(Oral potentially malignant disorders) and Group 2 (Oral squamous cell carcinoma). Thus, SMAD-4 can be considered as a prognostic factor in patients with Oral potentially malignant disorders and Oral squamous cell carcinoma.

From present study we came to an inference that SMAD-4 may play a vital role in tumorigenesis. In our study, SMAD-4 is found to be downregulated in OSCC in comparison to and OPMDS. A sequential upregulation of SMAD-4 expression is observed from OPMDS and then its sudden loss in OSCC suggesting a possible role of SMAD-4 in oral carcinogenesis. It may also act as a marker for early detection of malignant transformation with prognostic significance.

5. CONCLUSION

Besides the notion that SMAD-4 was a tumor suppressor and loss of SMAD-4 expression may lead to spontaneous oral squamous cell carcinoma development, patients whose oral potentially malignant lesions with higher levels of SMAD-4 expression displayed a

significantly higher rate of malignant transformation. Our results suggested that SMAD-4 might be activated in early oral tumorigenesis but insufficient to halt carcinogenic process. The combination of SMAD-4expressionand histological grade of dysplasia was a better predictor for the malignant transformation of oral leukoplakia.
However further larger studies are recommended to further validate its role in oral carcinogenesis.

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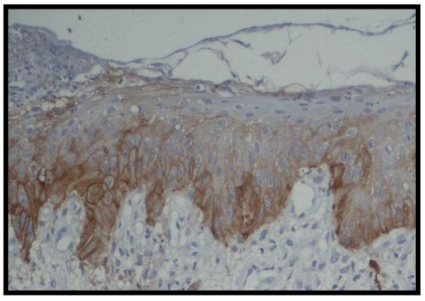
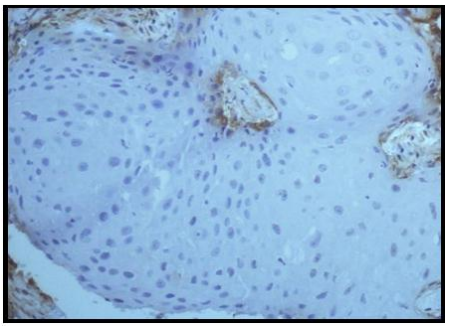
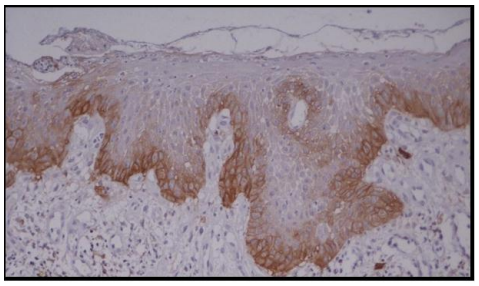


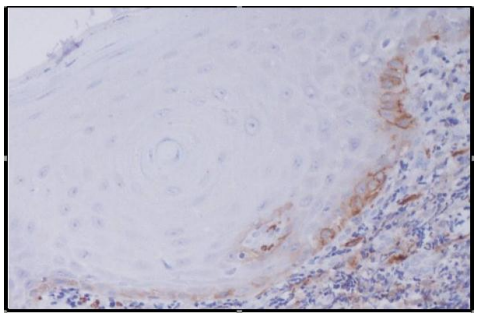
Photomicrograph 1: Immunohistochemicallystained section of SMAD-4 in Oral potentially malignant disorders showing moderate staining at 40x



Photomicrograph 2: Immunohistochemically stained section of SMAD-4 in Oral potentially malignant disorders showing mild staining at 40x



Photomicrograph 3: Immunohistochemically stained section of SMAD-4 in Oral squamous cell carcinoma showing negative staining at 40x



Photomicrograph 3: Immunohistochemically stained section of SMAD-4 in Oral squamous cell carcinoma showing mild staining at 40x