Original Article

Pathogenetic model of survivin-dependent molecular signalling pathways in tumorigenesis of oral cancer and precursor lesions

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Abstract

Background: p53 tumour suppressor gene limits unchecked cellular growth in response to DNA damage, by causing G1 arrest and the activation of apoptosis. Inhibitors of apoptosis include survivin which acts by inhibition of caspases. Survivin has a significant role as a cell cycle modulator and is only minimally present in mature tissues. Aberrant expression of p53 and survivin has been evaluated in various carcinomas. Thus, the objective of this research was to elucidate the co-expression of p53 and survivin in tissue samples of Oral Potentially Malignant Disorders (OPMDs) and Oral Squamous Cell Carcinoma (OSCCs).

Method: Thirty tissue samples of OPMDs and 30 tissue samples of OSCCs taken from department archives were used in the study. Expression of p53 and survivin was analyzed in the study groups by the help of immunohistochemistry. Also, co-expression of both the markers was evaluated.

Results: The expression of p53 and survivin in the oral epithelium of patients with OSCCs was significantly higher than that in patients with OPMDs (*P* value ≤ 0.05).

Conclusion: Our results provide insights into the altered survivin and p53 co-expression with significant immunoexpression within the study groups. Therefore, survivin and p53 could be better markers for identifying cell proliferation and apoptotic pathway. Also, malignant transformation rate of OPMD increases with increased expression of these markers.

Keywords: Apoptosis, cell cycle, immunoexpression, oral potentially malignant disorders (OPMDs), oral squamous cell carcinoma (OSCCs), p53, survivin

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INTRODUCTION

Oral cancer is among the most prevalent cancers. It affects 7% of women and 19% of men in India, respectively. The prevalence of smoking and chewing tobacco is accountable for India's high incidence of mouth cancer.[1] The death rates over the past 20 years have remained mostly stable despite advancements in cancer treatment and surgery, with a 5-year rate of survival that ranges between 35% and 50%.

One of the main causes of the poor prognosis of Oral Squamous Cell Carcinoma (OSCC) is the delay

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in diagnosis that drastically reduces the patient's life expectancy.[2] Increased genomic instability that involves activating oncogenes and turning off tumour suppressor genes leads to the development of oral cancer. Most human malignancies have biological mechanisms that control apoptosis, the balance between cell growth and death, and cell cycle progression in the early stages.[3]

The p53 gene is a multifaceted tumor suppressor gene located on chromosome 17p13.1. It produces a nuclear phosphoprotein that helps to limit unchecked cellular growth in response to DNA damage. This is achieved by causing G1 arrest and activating apoptosis.

p53 plays a crucial role in regulating gene transcription and monitoring cell cycle check points. It also helps to maintain genomic integrity by regulating DNA replication and repair. Mutations in the p53 gene are common in human malignancies and are a key driver of tumor development and progression.

In cases where the p53 gene is not mutated, other genes such as MDM2 and MDMX can control the activity of the p53 pathway by increasing the depletion of p53.[4]

The focus of recent research has switched to the class of proteins known as inhibitors of apoptosis (IAP) which includes the protein known as survivin that acts by inhibition of caspases. Wide expression of survivin is seen in foetal tissues, whereas in adult tissues it is minimally present or restricted expression is seen. During mitosis, interaction of survivin protein is noticed with mitotic spindle to maintain its integrity and further it eliminates cells by apoptosis with aberrantly formed mitotic spindles. Expression of survivin is also highlighted in cell cycle regulation. This gene is repressed in the G1 phase of the cell cycle and is highly expressed in G2/M. Cytoplasmic survivin is thought to be cytoprotective, whereas nuclear survivin is thought to regulate cell division and is expressed extensively in malignancies and is linked to the disease's aggressive behaviour. It is a key target for tumour diagnostics, prognosis, and anticancer therapy due to its selective expression.[5,6]

Survivin regulates mitosis in cancer cells by performing two major functions: (1) By joining with other proteins to create a chromosomal passenger complex and (2) by preventing apoptosis. Survivin combines to tubulin and localizes toward the mitotic spindle during mitosis, indicating that it is involved in the control of mitosis. Survivin is crucial for centrosome activities, microtubule formation at metaphase and anaphase, and spindle check points. Due to an arrest of DNA replication, survivin depletion results in aberrant cell division that activates spindle check points through the tumour suppressor protein p53.[7]

Survivin is present in abundant amount in intermitochondrial space and its translation to mitochondria may be associated to oncogenic transformation because, surprisingly, survivin is not found in mitochondrial fractions in normal cells. The mechanisms underlying survivin regulation are not fully understood, but several signaling pathways and factors have been reported to activate survivin in cancer cells, potentially promoting cell proliferation. In normal cells, wild-type p53 and retinoblastoma directly or indirectly repress survivin transcription. However, survivin is frequently overexpressed in cancer cells and is associated with mutations and functional losses in the retinoblastoma and p53 genes. Since, E2F activators can also induce survivin transcription, indicating that the retinoblastoma/E2F/p53 pathways may contribute to aberrant survivin expression.[8]

Preneoplastic and cancerous cells can grow clonally with a selective advantage, when the function of the gene p53 is impaired. Moreover, p53 mutation allows for upregulation of survivin which further helps in progression of the cell cycle and subsistence of cancer cells due to its antiapoptotic function. Thus, the present study was designed to investigate a possible correlation between the co-expression of p53-dependent and p53-independent survivin-mediated pathway immunohistochemically in Oral Potentially Malignant Disorders (OPMDs) and OSCC. Understanding of survivin-mediated and p53-mediated apoptotic pathways would aid the prognosis of oral potentially malignant disorders.

MATERIALS AND METHOD

Patients and tissue specimens

All the OSCC and OPMD patients' samples were taken from archival tissues from Department of Oral and Maxillofacial Pathology and Microbiology, between 2016 and 2021. Histopathologically confirmed cases of OSCC and OPMDs, as per the diagnostic criteria of World Health Organization 2005, were included. Complete clinicopathological data of the cases were retrieved. Patients who received any radiotherapy and/or chemotherapy, any concurrent disorder, immunocompromised, and suffering from debilitating disease were excluded. Among the 60 tissue specimens, 30 were of OPMD (included oral epithelial dysplasia) and 30 were of OSCC and were further categorized as Group I and Group II, respectively. Subgrouping was done into 10 cases each

of mild, moderate, and severe oral epithelial dysplasia for Group I and well-differentiated OSCC (11 cases) and moderately poor–differentiated OSCC (combined 19 cases) for Group II. All the tissue were subjected to immunohistochemical analysis. The institutional ethical committee approved the study (ITSCDSR/L/2020/015).

Immunohistochemistry

Paraffin-embedded formalin-fixed tissue sections of 4-micron thickness were taken. Survivin and p53 immunoexpression was analyzed. Sections were deparaffinized in graded xylene and alcohols. Antigen retrieval was done using 10 mmol/L Tris buffer at pH 9.0 and heated to cycles of 85°C for 5 minutes, 95°C for 10 minutes, and 98°C for 10 minutes. Immunohistochemical staining for protein was performed using avidin-biotin complex procedure with a streptavidin-biotin complex peroxidase kit. Primary Polyclonal Antibody anti-survivin (Biogenex Ind Pvt. Ltd., Catalogue number-ANB 26- 5M) and Primary Monoclonal Antibody anti-p53 (Biogenex Ind Pvt. Ltd., Catalogue number-AM 239- 5M) for 1 hour at room temperature along with secondary antibody-poly-Horseradish Peroxidase (HRP) secondary detection system (Biogenex Ind Pvt. Ltd) were used.

Immunohistochemical analysis

For survivin, urinary bladder was taken as positive control and for p53, OSCC served as positive control. Positive staining for survivin was seen as immunolocalization of brown color in the nucleus, cytoplasm, or both within the cell, and for p53, localization of brown color within the nucleus was seen. Expression was graded as positive and negative. The images of five high-power fields (40x magnification) were obtained using digital camera (Olympus EPL3) attached to the research microscope and was transferred to a computer system for analysis.

A. Quantitative analysis

For both p53 and survivin, immunostained sections were scanned to determine areas that were positively stained. At 40x magnification, five representative fields were selected and immunostained positive cells were counted separately for both molecules. A total of 1,000 (200/high-power field) cells were counted. By selecting five random fields which were not in continuum, a possible bias was minimized.

A ratio was also obtained by dividing the quantitative score of p53 by score of survivin for further analysis of specificity and sensitivity and preparing the case-control charts for identifying low-risk and high-risk cases.

B. Qualitative analysis

For survivin was done as per Nakagawa *et al*.[9]

For p53 as per Heath KG *et al*.[10]

Scoring was done as 0 for no staining, + for low intensity, ++ for moderate intensity, and +++ strong intensity.

C. Semiquantitative analysis

For survivin was done as per Muzio *et al*.[11] and graded as:

0 for <5% positive cells, 1 for 5%-25% positive cells, 2 for 26%-50% positive cells, 3 for 51%-75% positive cells, and 4 for >75% positive cells.

D. Immunolocalization for survivin was done as per Deo *et al*.[12] as:

0 for absent, 1 for Nuclear, 2 for cytoplasmic, and 3 for both nuclear and cytoplasmic.

For p53, nuclear expression was studied.

E. Topographical analysis

Immunoexpression of nuclear and cytoplasmic survivin and nuclear p53 staining was seen in the basal, para basal, and superficial cell layers of the epithelium in Group I and in peripheral and central cells in epithelial islands in stroma in Group II.

Statistical analysis

The SPSS program version 24 was used to examine the collected information. The mean and standard deviation were used to express the data. Pearson's Chi-square test was used to assess differences between the various variables. In addition, for both markers, the area under the curve values were obtained using the receiver operating characteristic (ROC) curve analysis. Case control chart was formed to analysis the high-risk and low-risk cases. P value ≤ 0.05 was considered significant.

RESULTS

The demographic details of the study groups are depicted in Table 1. For both immunomarkers, positive immunoexpression was seen in basal, parabasal and superficial cell layer in group I and in peripheral and central cells in group II [Figures 1 and 2]. Statistically significant results were obtained while comparing quantitative values

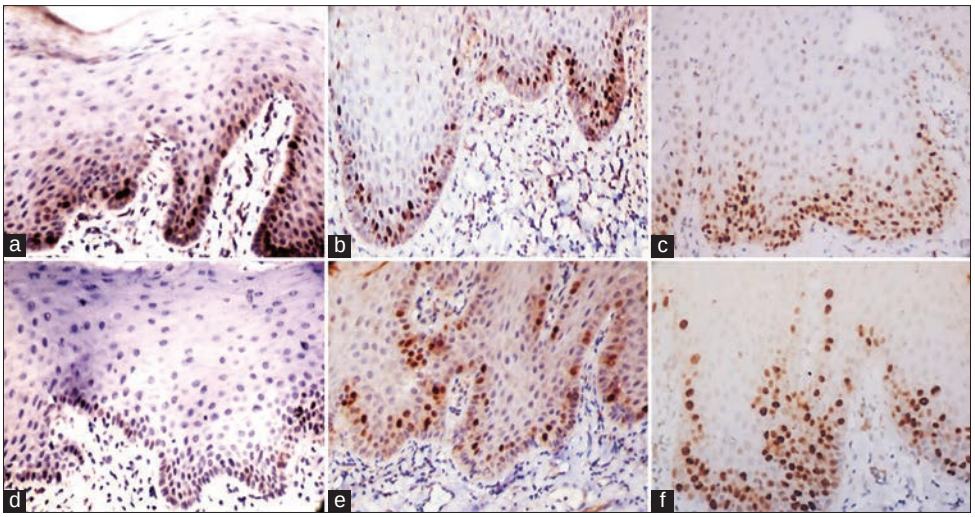

Figure 1: Immunopositivity of survivin seen as nuclear localization in basal and suprabasal cell layers in Mild Oral Epithelial Dysplasia (a), Immunopositivity of survivin showing both nuclear and cytoplasmic localization in basal and suprabasal cell layers in Moderate Oral Epithelial Dysplasia (b), Moderate immunopositivity of survivin showing nuclear localization in basal and parabasal cell layers in seen in a case of Severe Oral Epithelial Dysplasia (c). Mild immunopositivity of p53 showing nuclear localization in basal and suprabasal cell layers in Mild Oral Epithelial Dysplasia (d), Immunopositivity of p53 showing nuclear localization in parabasal cell layers in Moderate Oral Epithelial Dysplasia (e), Immunopositivity of p53 showing nuclear localization in basal and parabasal cell layers in Severe Oral Epithelial Dysplasia (f) (IHC, 40x magnification)

Table 1: Distribution of study cases according to demographic parameters

Demographic details		Group I (OPMD) Frequency (%) (*n*=30)	Group II (OSCC) Frequency (%) (*n*=30)	*P*
Gender	Male	21 (70%)	28 (93.3%)	0.020 (S)
	Female	9 (30%)	2 (6.7%)	
Age	<30 years	10 (33.3%)	0 (0%)	0.000 (HS)
	31-45 years	8 (26.7%)	2 (6.7%)	
	>45 years	12 (40%)	28 (93.3%)	
Site	Buccal Mucosa	25 (83.3%)	14 (46.7%)	0.006 (HS)
	Tongue	2 (6.7%)	1 (3.3%)	
	Alveolus	2 (6.7%)	14 (46.7%)	
	Vestibule	1 (3.3%)	1 (3.3%)	

S: Significant, HS: Highly significant

of p53 and survivin between the study groups. Quantitative mean value of p53 immunoexpression in group I was 43.72 ± 14.84, whereas it was increased to 93.98 ± 7.77 in group II. Similarly, mean ± standard deviation of survivin in group I was 50.34 ± 11.73, while 57.65 ± 9.82 in group II.

Moreover, on semi-quantitative assessment of survivin, maximum cases 60% in group I showed positivity in 5%-25% only, compared to 30% cases showing 76%-100% cell positivity in Group II. The results were statistically highly significant ($P \leq 0.05$).

Furthermore, maximum cases of group I (18 cases) and group II (12 cases) showed high intensity of survivin immunoexpression. On qualitative analysis, the immunoexpression and intensity of both p53 and survivin were seen in study groups [Table 2]. Moreover, direct correlation between co-expression of both markers which revealed that maximum cases showing co-expression for survivin and p53 were (26) 86.66% and (23) 78.88% in Group I and Group II. Additionally, a correlation between co-expression with histopathological grading was established. The results were statistically significant ($P \leq 0.05$ [Table 3]. Case-wise depiction of ratio of quantitative analysis of p53 and survivin in group I and group II. Of 60 cases, four cases were categorized as high risk [Figure 3a and b]. Furthermore, a correlation of p53 immunoexpression with survivin immunoexpression in group I and group II, respectively is depicted in [Tables 4 and 5].

Subsequently, ROC curve analysis, keeping p53:survivin ratio based on quantitative findings, reveals that the area under the curve was 76%. The sensitivity of the test was 69.2%. The specificity of the test was 68%. The cut-off value for transformation of case from group I to group II by ROC was 0.762 [Figure 3c].

DISCUSSION

Carcinogenesis is a multifaceted multistep process concerning the accretion of several alterations at genetic level. These modifications encourage progression of a normal epithelial cell to clinically apparent carcinomatous lesion proficient of invasion and metastases.[2] Almost all cancers are preceded by the development of a precancerous lesion, which then progresses to cancer at a later stage.[13]

Table 2: Assessment of Survivin and p53 immunoexpression in study groups

Immunomarker		Survivin			p53		
Parameters		Group I (OPMD) Frequency (%) (*n*=30)	Group II OSCC) Frequency (%) (*n*=30)	*P*	Group I (OPMD) Frequency (%) (*n*=30)	Group II (OSCC) Frequency (%) (*n*=30)	*P*
Expression	Absent	1 (3.3%)	4 (13.3%)	0.161	4 (13.3%)	5 (16.7%)	0.718
	Present	29 (96.7%)	26 (86.7%)		26 (86.7%)	25 (83.3%)	
Intensity	Weak (+)	2 (6.7%)	9 (30%)	0.035 (S)	15 (50%)	9 (30%)	0.293
	Moderate (++)	9 (30%)	5 (16.7%)		5 (16.7%)	4 (13.3%)	
	Strong (+++)	18 (60%)	12 (40%)		6 (20%)	12 (40%)	

S: Significant

Table 3: Histopathological correlation of study groups with immunoexpression of p53 and survivin co-expression

Characteristics	Study groups	Parameters	Survivin and p53 immunoexpression				*P*
			P53+/Survivin+	P53+/Survivin-	P53-/Survivin+	P53-/Survivin-	
Histopathological grade	Group I (OPMD) (*n*=30)	Mild (*n*=10)	10 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0.000 (HS)
		Moderate (*n*=10)	7 (23.3%)	0 (0%)	3 (10%)	0 (0%)	0.000 (HS)
		Severe (*n*=10)	9 (30%)	0 (0%)	0 (0%)	1 (3.3%)	0.002 (S)
	Group II (OSCC) (*n*=30)	WDSCC (*n*=11)	10 (33.3%)	0 (0%)	0 (0%)	1 (3.3%)	0.001 (S)
		MDSCC+PDSCC (*n*=19)	13 (43.3%)	2 (6.7%)	3 (10%)	1 (3.3%)	0.127

S: Significant, HS: Highly Significant, +: Present,-: Absent, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC+PDSCC: Moderately differentiated and Poorly differentiated squamous cell carcinoma combined

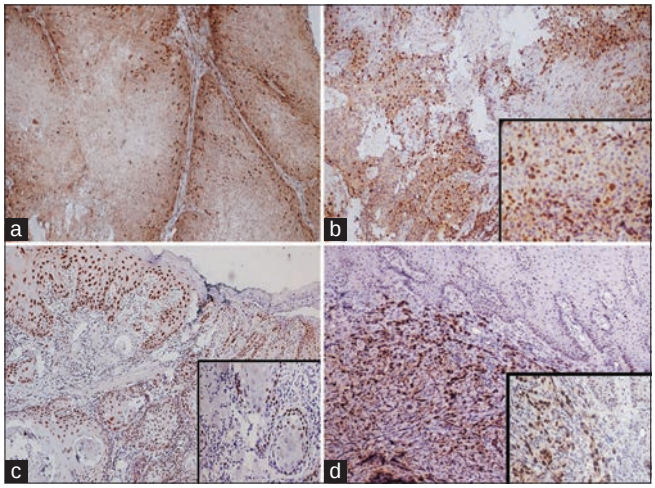

Figure 2: Immunopositivity of survivin (a) showing nuclear localization in peripheral cells of tumor islands in Well-Differentiated Oral Squamous Cell carcinoma (IHC,10x magnification magnification), Immunopositivity of survivin (b) showing nuclear localization in periphery of tumor cells arranged in form of cords in Moderately Differentiated Oral Squamous Cell Carcinoma (IHC, 10x magnification, Inset 40x magnification). Immunopositivity of p53 (c) showing nuclear localization in peripheral cell layers in Well-Differentiated Oral Squamous Cell Carcinoma, Immunopositivity of p53 (d) showing nuclear localization in individual tumor cells in Poorly Differentiated Oral Squamous Cell Carcinoma (IHC, 10x magnification, Inset 40x magnification)

In OSCC, biomarkers are genetic and molecular characteristics that are recognized as changed, amplified, overexpressed, silenced, or mutant genes and gene products. Several molecules potentially represent the loss of functioning tumour suppressors genes, cell cycle regulators, or apoptosis regulators, which results in an imbalanced cell growth and/or death process.[12] To recognize oral precancerous lesions prone to invasive transformation, new biological predictors of malignant development are required.

The IAP family of antiapoptotic proteins has homologues in vertebrate and invertebrate taxa, indicating that they are evolutionarily conserved. Due to their ability to functionally complement defects in the baculovirus protein p35, which binds to and inhibits caspases, two lAPs from the baculovirus family, Cp-IAP and Op-IAP, were the first to be identified. After that, several other human and Drosophila isoforms were found, and it has been shown that each one prevents cell death.[14] Biological functions of survivin are to serve both as a mitotic regulator and cytoprotective factor which include inhibition of apoptosis through several pathways and proper execution of mitosis and cell division. It can directly interact with caspases leading to the inhibition of caspase activity. Survivin expression was forced to counteract cell death produced by different apoptotic triggers, but survivin antisense or dominant negative mutants overexpressed in cancers and caused spontaneous apoptosis and numerous cell division abnormalities, including supernumerary centrosomes, multipolar mitotic spindles, and multinucleation.[15,16] As per the study done by Uren *et al.*,[17] endogenous or transfected survivin is linked to metaplate of the chromosomes and in the anaphase is linked to central spindle midzone.

Survivin is a helpful diagnostic biomarker of malignancy and a possible target for cancer treatment due to its expression in certain types of cancer and its significance in controlling cell cycle and preventing cell death.[18]

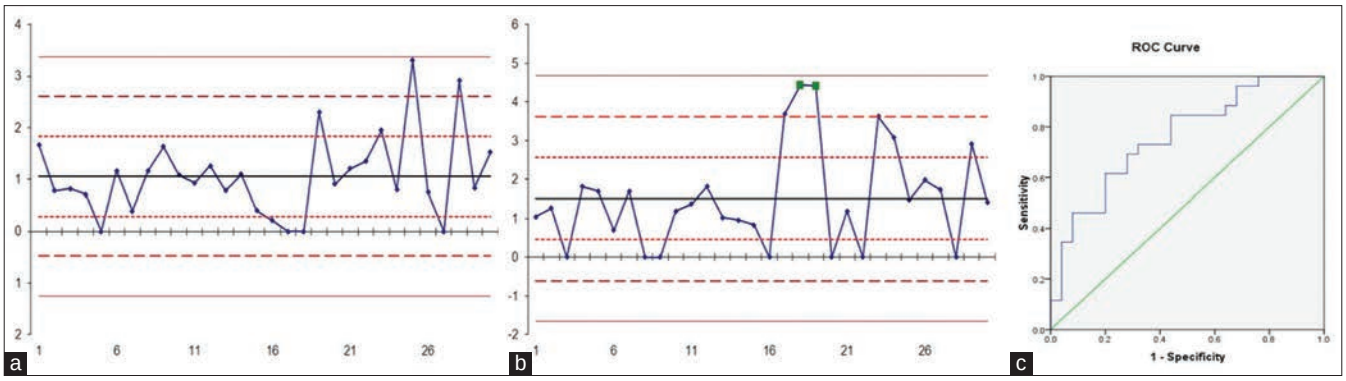

Figure 3: Ratio analysis of quantitative immunoexpression of p53 and survivin. (a) Showing case-wise analysis of Group I (OPMDs) depicting two high-risk cases of 30 cases, (b) showing case-wise analysis of Group II (OSCC) depicting two high-risk cases of 30 cases, and (c) depicting Receiving operator curve analysis showing area under the curve.

Table 4: Correlation of p53 immunoexpression with survivin immunoexpression in Group I (OPMD)

Parameters		p53 Expression		p53 Intensity				p53 Topography			
		Absent	Present	Absent	Weak	Moderate	Strong	Absent	B	B+SB	B+SB+SUP
Survivin Immunolocalization	Absent	1	0	1	0	0	0	1	0	0	0
	Nuclear	2	16	2	8	5	3	2	11	5	0
	Cytoplasmic	0	0	0	0	0	0	0	0	0	0
	Both	1	10	1	7	0	3	1	4	5	1
	P	0.03 (S)		0.095				0.128			
Survivin Intensity	Weak	0	2	0	1	0	1	0	1	1	0
	Moderate	2	7	2	4	2	1	2	6	1	0
	Strong	1	17	1	10	3	4	1	8	8	1
	P	0.02 (S)		0.344				0.241			
Survivin Topography	Basal	0	9	0	5	2	2	0	7	2	0
	B+SB	2	8	2	5	1	2	2	5	3	0
	B+SB+SUP	1	9	1	5	2	2	1	3	5	1
	P	0.04 (S)		0.466				0.142			

S: Significant

Table 5: Correlation of p53 immunoexpression with Survivin immunoexpression in Group II (OSCC)

Parameters		p53 expression		p53 Intensity				p53 Topography			
		Absent	Present	Absent	Weak	Moderate	Strong	Absent	Peripheral	Central	Both
Survivin Immunolocalization	Absent	2	2	2	0	2	0	2	2	0	0
	Nuclear	2	6	2	2	0	4	2	5	0	1
	Cytoplasmic	0	0	0	0	0	0	0	0	0	0
	Both	1	17	1	7	2	8	1	12	0	5
	P	0.07		0.04 (S)				0.196			
Survivin Intensity	Weak	0	9	0	2	2	5	0	6	0	3
	Moderate	1	4	1	0	0	4	1	2	0	2
	Strong	2	10	2	7	0	3	2	9	0	1
	P	0.169		0.009				0.208			
Survivin Topography	Peripheral	0	17	0	6	2	9	0	13	0	4
	Central	0	0	0	0	0	0	0	0	0	0
	Both	3	6	3	3	0	3	3	4	0	2
	P	0.01 (S)		0.02 (S)				0.061			

S: Significant

Survivin's differential expression makes it a desirable target for the selection of patients for molecularly based cancer therapy, and it can be employed as a prognostic indicator for proliferation and invasion.[12]

Exogenous survivin protein overexpression frees cells of p53-induced apoptosis, indicating that survivin depletion at least partially mediates the p53-dependent pathway. In reaction to stress signals, the tumour suppressor protein p53 encourages cell cycle arrest, ageing, and apoptosis, which plays a critical anticancer function. Ironically, both the protein and mRNA levels of survivin can be suppressed by wild-type p53. But in squamous cell carcinoma, more than 50% of cases are present with p53 mutations and are responsible for the overexpression of mutated survivin. It has been hypothesized that neither of the two potential

p53-binding domains in the survivin locus is required for the transcriptional suppression of survivin production despite their existence. The changed chromatin inside the survivin promoter was also proposed as a potential explanation for the p53-mediated silencing of the survivin gene.[7]

As per a prior study by Li *et al*.,[19] the expression of the protein survivin is either absent or very low in normal tissues, but it is selectively and intensely expressed in cancerous tissue, so this expression is tumour cell–dependent. Similar findings are seen in the current work, where survivin expression levels steadily rose as aberrant cell proliferation gave way to malignant transformation.

Therefore, the results of our study showed that among the study groups, survivin expression was positive in 29 (96.7%) cases of OPMDs and in 26 (86.7%) cases of OSCCs. In most of the cases in group I, 18 (60%) of the cases had nuclear localization, whereas in group II, 18 (60%) of the cases had both nuclear and cytoplasmic localization. Results were in accordance with the study done by Li *et al*.[20] where authors proved that nuclear and cytoplasmic subcellular pools of survivin appear to exist. This is in line with its role in controlling cell survival and cell division. One more hypothesis put forth that survivin's nuclear pool is typically engaged in encouraging cell proliferation and has been linked to poor prognosis in several immunohistochemistry investigations, whereas survivin's cytoplasmic pool may be involved in regulating cell longevity but not cell growth.[21] On the contrary, Thota *et al*.[22] findings suggest that there was no significant difference in serum survivin expression between oral cancer patients and noncancerous patients. The reason for this could be that survivin may be sequestered at the tumor site.

Furthermore, the findings of Li *et al*.[23] suggest that overexpression of survivin results in high activity of cell resulting in active formation of mitotic components like centrosomes and mitotic spindle microtubules during G2/M phase.

Moreover, 60% cases of group I and 40% cases of group II showed strong intensity of survivin immunoexpression. Semi-quantitatively, survivin analysis in the study groups revealed that many cases [18 (60%)] in group I exhibited 5%-25% positive cells, whereas majority of cases in group II (26.7%) (eight cases) showed 51%-75% positive cells. Similar results were seen by Kim *et al*.[24] where they showed that compared to other molecular markers such as Fas-ligand, Fas, and Bcl-2, survivin expression appeared to be more closely associated with the development of Cervical Intraepithelial Neoplasia and squamous cell carcinoma.

Further in our study, p53 immunoexpression reveals that in group I, 26 (86.7%) cases were positive, whereas in group II, 25 (83.3%) cases were positive for p53. The low concentration of p53 in normal cells could have explained the immune-negativity of p53 expression. Three potential explanations for this could have been: the absence of mutation, meaning the wild type p53 protein was undetected due to its short half-life; the mutation not resulting in protein stabilization; or the p53 gene being undetectable.[13] Depending on the cellular responses and kind of cell, p53 activation can cause several reactions. For instance, DNA damage may result in apoptosis or growth arrest, both of which aim to stop damaged cells from procreating and transferring mutations to the following generation. Because p53-deficient cells cannot effectively adapt to stress, they can have mutations that encourage the growth of cancer.[25]

In group I, maximum 15 (50%) cases had weak intensity of p53, whereas in group II, maximum 12 (40%) cases show strong intensity of p53. Similar results were seen by Mantovani *et al*.,[26] who suggested that quantifiable expression of p53 proteins might indicate the protein's stability via interactions with other intercellular proteins. In head and neck carcinomas, the expression of p53 frequently increased in tumorigenic phenotypes when the p53 gene was inactivated. These influences could have altered cellular activity.

Furthermore, histopathological correlation with immunoexpression of p53 and survivin co-expression revealed that maximum cases were positive for p53 and survivin in mild, moderate, and severe dysplasia. The same results were hypothesized by Gayathri *et al*.[27] that survivin protein accumulation might be an early event during step-wise malignant transformation and reflects the biologic aggressiveness of these lesions. Similarly, in group II, maximum cases were positive for p53 and survivin in moderately to poorly differentiated squamous cell carcinoma compared to well-differentiated OSCC**.** Kim *et al*.[28] proposed that survivin re-expression began in the early carcinogenesis phase (8 weeks), and there was a progressive rise in survivin translation throughout the development of oral carcinogenesis, as per immunohistochemical research.

CONCLUSION

Based on the results of our study, it can be inferred that elevated levels of survivin and p53 immunoexpression may constitute a key event in the early phases of carcinogenesis, thus providing a more accurate prediction for high malignant transformation rate of OPMDs. Thus, it can

be concluded that these markers exhibit the potential to serve as indicators of tumorigenic potential and apoptotic dysregulation in the initial stages of tumorigenesis. Activity of survivin is a key model in controlling cell proliferation and survival. Tumor cells employ complex evasion strategies to avoid being eliminated by the host immune response. Resistance to apoptosis is an important step in tumor cell evasion. Survivin expression is associated with altered sensitivity to antitumor drugs. Blocking of survivin function could emerge as a promising therapeutic intervention in oral carcinogenesis.

Ethical approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional standards. The study was approved by the Bioethics Committee of the Institution (No. ITSCDSR/L/2020/015). Informed consent was obtained from the patient.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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