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ORIGINAL ARTICLE

THE EFFECT OF SYNERGISTIC *Candida albicans* INFECTION WITH P63 PROTEIN EXPRESSION ON THE DEVELOPMENT OF ORAL CARCINOMA

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ABSTRACT

Oral cancer accounts for 2-4% of all cancer cases. There is compelling evidence that suggest that *Candida* may influence the development of oral cancer by interfering with the expression of the Tp63 gene, which is crucial for tumor progression, and promotes proliferation. The goal was to assess the p63 expression, compare it qualitatively and quantitatively in a normal buccal mucosa, epithelial dysplasia, leukoplakia, and oral squamous cell carcinoma (OSCC), and also determine how does it relate to the *Candida albicans* infection. A retrospective cross-sectional study of 100 Formalin-Fixed Paraffin-Embedded (FFPE) cases and controls were studied, and they were divided into four groups: Group I, included 40 controls with normal oral mucosa; Group II, included 20 cases of dysplasia; Group III, included 20 cases of leukoplakia, and Group IV, included 20 cases of OSCC. *C. albicans* was examined by Real-Time PCR based on *Erg11* gene and the immunohistochemical expression of p63 protein. Significant overexpression of p63 and positive PCR results of *C. albicans* were seen in noninvasive lesions (leukoplakia, epithelial dysplasia) and invasive OSCCs with an increased suprabasal expression in cases of leukoplakia and epithelial dysplasia. The mean of labeling index (LI) of p63 with *C. albicans* positive cases were found to be in increasing order from normal oral mucosa than leukoplakia and dysplasia to OSCC. These findings point to p63 association with oral carcinogenesis and candidal infection, and they also imply that dysplastic and leukoplakia lesions may have the ability to serve as a signal for oral premalignancy due to increased LI and higher suprabasal expression of this gene.

KEY WORDS: *Candida albicans*; epithelial dysplasia; *Erg11*; leukoplakia; p63 protein.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a type of cancer that is commonly referred to mouth cancer. It arises in the oral cavity's lining of the lips, mouth, upper throat, and 44 other locations. The OSCC is the primary cause of death and the sixth globally most common cancer in terms of reported cases. For men, it ranks as the sixth most common cancer, while for women, it ranks 12th most common. Age groups, genders, and races all have different yearly incidence and mortality rates and approximately 62% of cases begin in developing nations. According to the Iraqi cancer registry OSCC accounts for about 91.5% of all oral cancer cases, 37% of head and neck cancer, and 10% of all deaths across all age groups, and 4.5% of all cancer cases (Alshami et al., 2023).

While there are risk factors for alcohol and tobacco use, there is also a risk factor for Human Papillomavirus (HPV) infection. However, little is known about the potential role of HPV in the oral cancer development, despite studies showing that HPV can immortalize oral keratinocytes in vitro and histologic similarities between lesions of the vaginal and oral mucosa (Muhsin & Hadi, 2019). Furthermore, a strong body of evidence indicates that *Candida* may have an impact on the development of oral cancer (Abati et al., 2020; Di Cosola et al., 2021). However, no study has thoroughly examined this phenomenon of underlying mechanisms that *Candida* may encourage the growth and progression of OSCC.

Oral candidiasis is also more common in patients undergoing radiation therapy for head and neck cancer, which gives credibility to the connection between oral yeast dysbiosis and OSCC (Theofilou et al., 2022). When the epithelial barrier is damaged or malfunctioning, *Candida* species, especially *C. albicans*, the most prevalent yeast in the oral cavity, may proliferate uncontrollably and penetrate host mucosal tissues by the development and secretion of fungal hydrolytic enzymes, hypha formation, and touch sensing (Arita et al., 2022). Additionally, it was discovered that the development of oral cancer was associated with the virulence characteristics of *Candida* and the ability to produce acetaldehyde derived from ethanol (Alnuaimi et al., 2016). Furthermore, increased hydrolase production may cause chronic inflammatory responses in the host tissues, and the *Candida*'s high biofilm-forming ability may guarantee the long-term exposure of host tissue to fungal carcinogens like acetaldehyde (Alnuaimi et al., 2016), also one of the risk factors for the development of tumors is persistent inflammation brought on by microbial infection.

There is growing evidence in supporting the theory that oral candidiasis increases the rate at which oral tumor progression occurs in patients (Abati et al., 2020). Subsequently, the *Tp63* gene (or just p63) produces the tumor protein p63 as a transcription factor that binds to particular DNA sequences to

control the expression of particular genes (Vadovics et al., 2022). The timing of the on and off switch for many different genes is controlled by the p63 protein through interactions with other proteins. The activity of p63 (apoptosis) regulates a variety of cellular processes, including cell adhesion, differentiation, and growth and division (proliferation) (Rodriguez Calleja et al., 2022). Since p63 promotes survival and proliferation in tumor cells, it is believed to be essential for the self-renewal of tumors. When overexpressed, this protein may serve as a marker for cancer cells to determine the aggressiveness of OSCC. In comparison to uninfected tissues, a recent analysis of OSCC *in vitro* (xenograft samples) revealed that *C. albicans* increased *Tp63* expression and localization to the nucleus. It also had effects on the epithelial-mesenchymal transition (EMT), which is essential for cancer cell invasion and metastasis. Furthermore, in oral candidiasis-OSCC xenograft samples (OC-OSCC), thrombosis was also found. Therefore, it is known that thrombosis and tumor invasion are positively correlated, but the specific pathological mechanisms are unknown (Badwelan et al., 2023).

Due to the fact that premalignant conditions like leukoplakia, erythroplakia, and oral submucous fibrosis (OSMF) often precede OSCCs. At the same time, it is exceedingly challenging to predict when these oral PMDs will turn malignant. Therefore, it would be very beneficial to identify any early genetic alterations in these lesions, making it easier to identify lesions that could eventually progress to OSCC. This indicates that *Tp63*'s role in the oral epithelium may be to preserve stem cell function as opposed to the direct association between oncogenesis and malignant transformation. As a result, *Tp63* may play a crucial part in the potentially malignant disorders (PMDs) neoplastic transformation. Even though there is a wealth of data showing a direct correlation between *Tp63* and many aspects of epithelial cancers, the underlying molecular mechanisms and the full repertoire of the downstream target genes, by which *Tp63* promotes and contributes to the development of OSCC, are not fully known.

Consequently, the purpose of this investigation was to assess p63 expression in OSCC and determine how it related to key clinic-pathological variables and microbial infection (Steurer et al., 2021; Rodriguez Calleja et al., 2022).

MATERIAL AND METHODS

Ethical approval

This study was authorized by the Al-Nahrain University College of Medicine's institutional review board (I.R.B.) on November 11th, 2021, under the code number 59.

Sampling

The current study was designed as retrospective (case control) study, one hundred archival Formalin-Fixed Paraffin-Embedded (FFPE) cases and controls which were divided into 40 controls of normal buccal mucosa (Group I), 20 cases of epithelial dysplasia (Group II), 20 cases of leukoplakia (Group III), and 20 cases of OSCC (Group IV). The cases and controls were selected from the department of histopathology of teaching laboratories and Ghazy Al-Hariri Hospital for surgical specialties that belongs to the Baghdad Medical City and Forensic Hospital Baghdad respectively, after reviewing the Hematoxylin and Eosin (H&E) stained sections. As the following, 4 μm thick sections were made on ordinary slides to be subjected to H&E stain which have conducted to confirm the diagnosis of cancer types, stages and grades and for the purpose of conducting immunohistochemistry procedure to detect the presence of *TP63* other 4 μm thick sections were made onto positively charged slides and 25 mg sections of FFPE for DNA extraction which is subjected to the Real-Time PCR technique to detect the presence of *C. albicans* based on *Erg11* gene. Positive control tissue for immunohistochemical (IHC) procedure was prepared from tissues that were previously known to contain the target marker (TP63) they included prostate tissue.

Immunohistochemical assay for TP63

According to the manufacturer's instructions, the manufacturer's product #E-IR-R213A from Elabscience Biotechnology Inc. USA ready to use, was immunohistochemically stained for the expression of p63. In a nutshell, glass slides coated with poly-l-lysine and displayed with tissue sections that were cut at a thickness of 4 μm. Epitope retrieval solution (Leica 10×, pH 9) was used with microwave to boil temperature once over a period of 20- and 30-min.

The 2-step Plus is a two-step, broad-spectrum immunohistochemical detection tool that can directly increase the binding signal of an antibody to an antigen. This approach successfully avoids the space-steric barrier brought on by too many polymer molecules while simultaneously maintaining the antibody's capacity to attach to antigens specifically. The primary antibody (6 mL) is a mouse-derived monoclonal antibody with the designation PA6082® from Elabscience Biotechnology Inc., ready to use. Better binding primary antibody detection is made possible by the special polymer auxiliary agent for macromolecular detection system. This kit includes a diluent for the concentrated DAB solution to counteract the effects of varying water alkalinity and acidity on the DAB chromogenic agent.

The slides were then displayed, counterstained for 2 min with Harri's hematoxylin, and all batches of study sample staining were conducted along with positive and negative staining controls. Positive controls included healthy human prostate tissues that had been shown to react antigenically to p63 in prostatic epithelial basal cells. It was possible to perform a negative control by skipping the primary antibody stage (Elabscience Biotechnology Inc.).

Evaluation of the TP63-IHC signals

The results were assessed for presence of p63 signals, percentage of scoring positive staining cells (LI; labeling index) and pattern (localization of staining) of p63 expression. Immunohistochemical staining of positive control (prostate epithelium) and negative control were also considered. The grading was performed by two observers independently to prevent interobserver bias. The slides were then mounted, counterstained for 2 min with Harri's hematoxylin, and all batches of study sample staining were conducted along with positive and negative staining controls. Positive controls included healthy human prostate tissues that had been shown to react antigenically to p63 in prostatic epithelial basal cells. It was possible to perform a negative control by skipping the primary antibody stage (Sinha et al., 2015).

Counting criteria

Under a light microscope, stained sections were examined and evaluated. Cases demonstrating a consistent brown coloring of DAB in the nuclei of epithelial cells at low power were regarded as positive. Negative sections were those that completely lacked epithelial cell immunostaining (Sinha et al., 2015).

At a magnification of 200×, the positivity of individual cells was determined. Cells that had uniform brown nuclear staining were deemed positive, whereas cells that displayed only hematoxylin staining were deemed negative (Sinha et al., 2015).

A qualitative assessment (semi-quantitative) based on the degree of p63 positive in the basal/ parabasal, suprabasal, and superficial layers of normal oral epithelium, various stages of oral epithelial dysplasia, and leukoplakia was performed at a magnification of 400×. Based on the degree of staining of tumor islands, the pattern of expression in OSCC was classified as peripheral, central, or diffuse. The positive results were then rated for statistical analysis based on staining intensity. Based on a study by Castle et al., the expression in cases with staining heterogeneity was categorized as (+) light staining, (++) moderate staining, and (+++) intense staining according to the prevailing staining intensity. To remove interobserver bias, two additional observers conducted each observation (Patel et al., 2017; Sinha et al., 2015).

Analyzing the labeling index (LI) quantitatively, by selecting three non-overlapping fields and calculating the positive and negative stained cells using an Olympus microscope under the 400x and 100x magnification and photomicrographs were obtained. The average percentage of positive cells was used to express the results. If a specimen had 10% or fewer of the positive cells, it was considered negative, 10%–25% weakly positive, 26%–75% moderately positive, and 76%–100% strongly positive (Bortoluzzi et al., 2004; Mitildzans et al., 2017).

DNA extraction and quantification

The 25 mg of the biopsy was used to extract DNA using the gSYNCTM DNA® Extraction Kit (Geneaid Biotech Ltd.) following the manufacturer's instructions. The kit lysed cells and broke down protein using proteinase K and chaotropic salt, which made it possible for DNA to attach to the spin column's glass fiber matrix. Water was used to elute the purified genomic DNA after contaminants were eliminated using a wash buffer. Gel electrophoresis was used to detect the genomic DNA's integrity by Using a Nanodrop, the extracted DNA's concentration and purity were determined by inserting 5 µl of extracted DNA into the instrument sample cell. The extracted DNA with an approved absorption wavelength of 260/ 280 and a purity of 1.8-2 would be suitable for using.

Identification of ERG11 gene of C. albicans by Real-Time PCR

The *Erg11* gene of *C. albicans* was amplified by using primers and probes which were designed previously by Chau et al. (2004), as follow: probe (FAM) TGCCTGACCCTGATTATAGTTCAATGGTGG, primer forward AACTACTTTTGTTTATAATTTAAGATGGACTATTGA and primer reverse AATGATTTCTGCTGGTTCAGTAGGT. Reagent volumes were calculated using the TaqMan 2× PCR Master Mix manufacturing process (Norgen Biotek Corp Copyright©2023, Canada; # 28340). Which is a ready-to-use solution that contains a PCR internal control that can be detected by a real-time PCR machine's HEX/VIC channel. Users can validate the DNA template quality and avoid any false negatives in the PCR results by spotting the internal control. To set up the TaqMan real-time PCR, only the template, target TaqMan primer/ probe combination, and water are required, besides based on the number of controls and samples plus one more reaction to insure a sufficient volume. The thermal cycling conditions were: initial denaturation at 95 °C for 10 min (1 cycle), followed by (35 cycles) of denaturation at 95 °C for 15 seconds and 61 °C for 30 seconds for annealing and extension. These processes were carried out on a CFX96 BioRad apparatus. As a positive control, *C. albicans* ATCC 10231 strain was utilized, and water was used as a negative control and internal

controls were used. In the final step of the thermal protocol, the CFX96 Bio-Rad device instrument software automatically calculates the baseline cycles and threshold, and the amplification curve was given for each sample as the (Y) axis is the Ct – Threshold cycle, and the (X) axis the fungal or the housekeeping DNA copy number. The screen in Report mode presented qualitative results. The housekeeping gene was examined for those undetectable samples in order to prevent providing false negative results. Samples that crossed the threshold in the channel (FAM) are displayed as positive while those that did not cut the threshold were reported as negative or (NO CT).

Statistical Analysis

To ascertain whether there was a correlation between *C. albicans* and p63 protein expression with study groups, data were evaluated using means, standard deviations, and tables of frequencies. Using SPSS statistical software, version 25, a Chi-square test was run with a significance level of p value 0.05 (Pandis, 2016).

RESULTS

Four groups were created using samples of patients out of all the cases classified in the following groups. Normal oral mucosa (I) 40 (40%) and 20 (20%) for each epithelial dysplasia (II), leukoplakia (III), and OSCC (IV). Chi-square test and analysis of variance were used in the statistical analysis (ANOVA) 0.05 or less was regarded as significant.

Table 1. Qualitative assessment and mean labeling index of p63 expression in the samples from patients in the groups: normal buccal mucosa (I), dysplasia (II), leukoplakia (III) and OSCC (IV).

Group	No expression	Expression	MD ± SD*	Total	$\chi 2$ (p)
I	16 (16%)	24 (24%)	36.30 ±13.408	40 (40%)	13.170 (0.004)
II	2 (2%)	18 (18%)	61.06 ±17.531	20 (20%)	
III	3 (3%)	17 (17%)	48.61 ±9.580	20 (20%)	
IV	1 (1%)	19 (19%)	63.11±23.769	20 (20%)	
	22 (22%)	78 (78%)	49.67 ±19.881	100 (100%)	

$\chi 2$ = Chi-square test (degree of freedom= 3); $p < 0.05$ significant. *Mean ± Standard deviation

Twenty-two cases had no p63 expression, while 78 (78%) had positive signals for the p63 protein, as shown in Table 1, where expression was detected in the basal layer of normal epithelium as well as the suprabasal layer (Figures 1A and 1B). In normal oral tissues, the mean labeling index (LI) of p63 ranged from 36.30 ± 13.408, with the mean LI of OSCC being 63.11 ± 23.769.

Table 2. Positive cells (%), staining intensity and patterns for noninvasive and invasive lesions of p63 expression.

IHC-Scoring criteria Normal		Group				p value
		Dysplasia	Leukoplakia	OSCC		
Labeling index (n)	Weak	17	1	4	3	< 0.001**
	Moderate	7	11	13	5	
	Strong	0	6	0	11	
Staining intensity (n)	+	8	2	3	6	0.01*
	++	15	11	10	8	
	+++	1	5	4	5	
Patterns of expression in Dysplasia and leukoplakia (%)	Basal	18	2	2	0	< 0.001**
	Suprabasal	6	13	13	0	
	Superficial	0	3	2	0	
Patterns of expression in OSCC (%)	Central	0	0	0	3	
	Diffuse	0	0	0	8	
	Peripheral	0	0	0	8	

* Significance level of p value< 0.05. ** High significance level of p value <0.001. IHC: immunohistochemical

Table 2 provides an overview of the LI, patterns, and intensity of p63 expression in the groups of oral tissues. For p63, all test specimens were positive. Basal, parabasal, and suprabasal cells of normal mucosa had moderate p63 staining intensity, which gradually faded at the midlevel of the epithelium (Figure 1A). Suprabasal keratinocytes consistently stained with p63 in every abnormality, and this staining could not be differentiated from healthy mucosal staining.

The percentage of p63 (LI)-positive cells is also displayed in Table 2. When the percentage of p63-positive cells was compared between the normal mucosa staining and either the dysplasia or the carcinomas, statistically significant differences were identified. The bulk of the tumor cells in the carcinoma displayed dispersed and peripheral p63

staining (Figure 1D). A significant difference between the patterns of p63 expression in OSCC was revealed when the percentages of p63-positive cells of invasive and non-invasive lesions were compared. Dysplastic epithelium displayed p63 expression in the basal and suprabasal layers, with a little expression in the superficial layers (Figure 1C). In cases of leukoplakia, the suprabasal layers showed the most p63 expression. In two of the cases, the expression was visible in the topmost layers (Figure 1D). In OSCC, three staining patterns were seen. Just the central layer of tumor islands expressed p63 in a small number of cases. P63 expression was occasionally seen in dispersed and peripheral cells (Figure 1D).

Table 3. P63 expression and PCR results for the *Erg11* gene in *Candida albicans* in the samples from patients in the groups: normal buccal mucosa (I), dysplasia (II), leukoplakia (III) and OSCC (IV).

Markers		Groups				
		I	II	III	IV	p value
P63	Expression (n)	24	18	17	19	0.004*
	No expression (n)	16	2	3	1	
Erg11	Positive (%)	10	17	12	15	< 0.001**
	Negative (%)	30	3	8	5	

* Significance level of p value< 0.05. ** High significance level of p value <0.001

In Table 3, a significant positive Real-Time PCR results of *Erg11* gene of *C. albicans* among the groups were found with high positivity in dysplasia's and carcinoma's as these showed high expression of p63 protein.

Regarding to the IHC- Scoring criteria of p63 existence, percent of positive cells LI, intensity of reaction and patterns of p63 expression with Candidal infection a significant association among them were found; most of the positive *C. albicans* cases were with highly p63 protein expression, moderate scoring of positive cells, moderate intensity of reaction and suprabasal pattern of p63 expression as indicated in Table 4.

Table 4. Association IHC-Scoring criteria for study groups of p63 expression with Real-Time PCR results of *Erg11* of *Candida albicans*.

IHC-Scoring criteria Positive		Real-Time PCR results of *C. albicans*		p value
		Positive	Negative	
P63 expression	Expression	49	29	0.01
	No expression	5	17	
Labeling index (LI)	Weak	13	12	0.004
	Moderate	23	13	
	Strong	13	4	
Staining intensity	(+)	13	6	0.002
	(++)	24	20	
	(+++)	12	3	
Patterns of expression in dysplasia and leukoplakia	Basal	12	10	0.02
	Suprabasal	19	13	
	Superficial	4	1	
Patterns of expression in OSCC	Central	3	0	
	Diffuse	5	3	
	Peripheral	6	2	

* Significance level of p value< 0.05. IHC: immunohistochemical

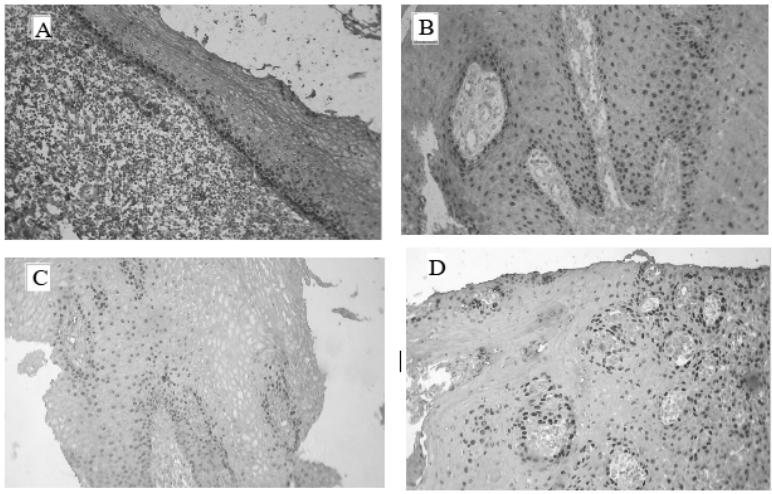

Figure 1. Photomicrograph showing p63 expression in oral tissues. (A) p63 expression in normal control: strong basal expression and intense reaction was seen (10×). (B) p63 expression in dysplastic lesion moderate basal, Suprabasal expression was seen (10×). (C) p63 expression in leukoplakia lesion basal, Suprabasal expression was seen (10×). (D) p63 moderate expression in OSCC lesion central expression was seen (10×).

DISCUSSION

Lesions of the oral mucosa, whether it is in a malignant stage like oral squamous cell carcinomas or a premalignant stage like potentially malignant oral diseases (PMOD), may attack any tissue in the oral cavity (Hadi et al., 2020), according to the World Health Organization (OSCC). A few examples of PMODs are leukoplakia, erythroplakia, actinic cheilosis, and oral submucosal fibrosis (Bouaoud et al., 2022) with incidence rates ranging from 0.02 to 0.83%, erythroplakia is less frequent than leukoplakia, yet some of them have been labeled as microscopic dysplasias. Its estimated prevalence by 4.1%, with malignancy developing in 1 to 18% of cases (Walsh et al., 2021).

The SCCs have been evaluated in studies with the main goal of determining the biology, severity, and prognosis of oral malignancies with a variety of behaviors. Since it is unclear how *C. albicans* infections and p63 protein expression in premalignant lesions affect malignant transformation, so the clinical and histological research are required. Because of these lesions might be reversed with an early diagnosis and a prompt infection treatment, therefore these relationships may have clinical relevance (Di Cosola et al., 2021; Walsh et al., 2021).

Furthermore, in normal and non-dysplastic mucosa, p63 expressed in basal and parabasal keratinocytes of the epithelium, with Np63 serving as the primary functional isoform. As dysplasia improves, the percentage of p63-positive cells increases and extends into the upper epithelial layers. Additionally, overexpression of p63, particularly Np63 which increased p63 expression and inflammatory cell infiltration and oral leukoplakia has a worse prognosis and a faster rate of cancer progression (Melino, 2011; Rodriguez Calleja et al., 2022) as shown in Table 1, where expression was detected in the basal layer of normal epithelium as well as the suprabasal layer (Figures 1A and 1B), with different percentages of p63 expression in dysplastic mucosa, leukoplakia and OSCC.

The opportunistic pathogen *Candida* fungus is frequently found in the mucous membranes of the oral cavity, and it is more active in people who have compromised immune systems (Rapala-Kozik et al., 2023). The most researched *C. albicans* strain is a risk factor for premalignant lesions in the oral cavity that develop into malignant lesions, and it was detected with high percentages in dysplastic oral tissues, leukoplakia and OSCC as shown in Table 3. Moreover, a number of processes, including the generation of nitrosamine and acetaldehyde, have been linked to histological alterations like epithelial hyperplasia, hyperkeratosis, microabscesses, and chronic inflammation.

Considering that cancer is a progressive disease that proceeds from premalignant lesions to malignant stages, it is imperative to develop prognostic strategies in the early stages of the disease caused by viral and fungal infections. These infections may cause tissue dysplasia linked to the development of cancer (Muhsin et al., 2019). A review of the literature revealed that only a small number of research have examined the expression of p63 in leukoplakia, dysplasia, or OSCC with Candidal infection. In addition to identifying *C. albicans,* this study also looked at p63 expression in leukoplakia and OSCC. When leukoplakia patients progressed from mild to severe dysplasia, both *Erg11* and p63 expression increased (Vadovics et al., 2022) as presented in this study.

There have been reports of p63 overexpression in adenocarcinoma and SCC, adenoid carcinoma, polymorphous low-grade adenocarcinoma, basal cell and canalicular adenomas, esophageal carcinoma, and prostate cancer. P63 expression was mostly observed in the peripheral cells of tumor nests in the well-differentiated tumor region, which is similar to a previous study (Patel et al., 2017) as seen in (Figure 1D) from this study. Since some lesions may emerge even in the absence of significant risk factors like alcohol and tobacco use, the presence of infectious organisms in the oral cavity has become more important in the study of pre-malignant and malignant lesions. A few pathogenic microorganisms, such as *C. albicans,* HPV and Epstein-Barr virus (EBV) can also disrupt cellular processes and result in histological abnormalities in the tissues of the oral cavity. Oral leukoplakia, one of the most

common conditions of the oral cavity, has a 4.1% likelihood of developing into cancer, primarily affecting the tongue. The base of the mouth and the tongue were, in fact, the anatomical region most frequently damaged in the current study. Moreover, leukoplakias have been reported on the tongue, the gingiva, the dorsolateral side of the tongue, and the buccal mucosa (Erira et al., 2021; Peng et al., 2022)

Kristoffersen et al., 2012 mentioned statistics show that, despite older individuals having a higher prevalence of leukoplakia diagnoses, early detection is challenging since there is insufficient clinical examination follow-up and no symptoms. Nonetheless, it has been demonstrated that the disease's natural history is one that is progressive and curable in light of the evidence.

It has been noted that oral leukoplakia frequently has *C. albicans* infection, which is a significant risk factor. Despite the fact that it concerns the public's health, the relationship between infection and leukoplakias is still up for debate. This fungus may enter the outermost layers of the epithelium, and the majority of strains engage in a process called commutation that enables the fungus to transform into new phenotypes with distinct morphological and functional characteristics. This could then lead to the histological changes that have been observed in other investigations and reported in the current investigation (Di Cosola et al., 2021; Li et al., 2022). Histopathological alterations like epithelial hyperplasia, hyperkeratosis, microabscesses, and chronic inflammation have been linked to several pathways, like the formation of nitrosamine and acetaldehyde. This work, however, highlighted the modest histological alterations associated with *C. albicans Erg11* positivity that warrant more investigation in the future.

As a result, this study demonstrated that oral premalignant and malignant lesions express p63 substantially more than healthy oral mucosa does. Epithelial dysplasia, leukoplakia, and OSCC were the conditions with the highest levels of p63 LI. This finding suggests that the increased p63 expression in the oral epithelium may function as a marker for premalignancy. Increased p63 expression in the oral epithelium is thought to be indicative of increased proliferative potential and aberrant maturation, both of which would predispose to malignant transformation. The most prevalent form of *C. albicans* infection was oral dysplasia, which is associated with dysplastic diseases when p63 is expressed more strongly.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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