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Original Article

Association of oral tumor suppressor gene deleted in oral cancer-1 (*DOC-1*) in progression of oral precancer to cancer

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ABSTRACT

Introduction: Deleted in oral cancer-1 (DOC-1), a highly conserved tumor suppressor gene, encodes a specific cyclin-dependent kinase 2-associated protein 1 ($p12^{DOC-1}$). The protein DOC-1 arrests the cells in the G_1 phase of the cell cycle and regulates DNA replication in the S phase of the cell cycle. It is known that DOC-1 is downregulated in head and neck cancer. Hence, the aim of the study was to analyze the messenger RNA (mRNA) expressions of DOC-1 in patients with oral lichen planus (OLP), leukoplakia (LPK), oral submucous fibrosis (OSF), and oral squamous cell carcinoma (OSCC) in comparison to normal control subjects, and these were correlated with differential oral habits and duration of exposure.
Materials and methods: Semiquantitative one-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to assess the potential role of DOC-1 in differential oral conditions.
Results: The expression of DOC-1 at the transcriptional level was found to be consistently reduced (39.13%) or non-detectable (60.87%) in OSCC cases; LPK cases also showed reduced (26.19%) or non-detectable (59.52%) DOC-1 expression, and overexpression of DOC-1 was observed in patients with OSF (42.3%) and OLP (35.29%) as compared to the normal control groups. A marked reduction in DOC-1 expression was observed in patients who smoke *bidi* and chew tobacco compared to patients who smoke cigarettes and chew *pan*. An overall reduced expression of DOC-1 with an increase in the duration of exposure was observed.
Conclusions: The overexpression of DOC-1 in oral premalignant disease groups with reduced or loss of expression in OSCC groups suggests its association in the progression of oral carcinogenesis, suggesting DOC-1 to be an important prognostic indicator for oral cancer.

1. Introduction

Oral cancer (OC) has high mortality and morbidity rates as most patients are presented for treatment at late stages of the cancer. It has been well established that oral squamous cell carcinoma (OSCC) manifests as several biochemical, cellular, and clinical changes in the affected epithelium of the oral mucosa. Sometimes, this is preceded by white, red, or mixed mucosal changes, and these are known as oral potentially malignant disorders (OPMDs) such as oral lichen planus (OLP), leukoplakia (LPK), and oral submucous fibrosis (OSF) [1–3]. The malignant transformation rate for OLP was 1.1–1.63% [4,5], LPK 0.13–17.9% [6,7], and OSF 7.6% [8]. Habitual usage of areca quid/*pan*, *bidi*/cigarettes, and commercially available smokeless form of products such as *gutkha*, *pan masala*, *khaini*, and *supari*, has been considered as the main etiological factor for OPMDs and OC [9,10]. Cell cycle machinery deregulation is a fundamental hallmark of cancer progression [11]. Deregulations in cell cycle regulatory protein are common in oral carcinogenesis [12]. The abrogation of cell cycle regulatory proteins such as retinoblastoma tumor suppressor protein (pRB), cyclin D1, cyclin-dependent kinase (CDKs), and CDK inhibitors ($p12^{WAF1/CIP1}$, $p27^{KIP1}$, and $p16^{INK4a}$) occurs in response to extracellular/intracellular stress and DNA damage, and these proteins flock on the cell cycle machinery and stimulate oral carcinogenesis [12].

Recently, cell cycle regulatory proteins such as CDK2AP1 (cyclin-dependent kinase 2-associated protein 1; $p12^{DOC-1}$) corresponding to the gene DOC-1 (deleted in oral cancer 1) have been found

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to be extensively involved in the pathogenesis of several cancers. DOC-1 is a highly conserved tumor suppressor identified by normal hamster oral keratinocytes by suppression subtractive hybridization experiments [13]. DOC-1 is ubiquitously expressed and highly conserved; the gene is mapped on chromosome 12q24, and a complementary DNA (c-DNA) 1.6 kilobase pairs (kb) in length has been cloned from humans, mice, and hamsters [13–15]. DOC-1 was revealed to have three recognized binding targets DNA polymerase alpha/primase [16], DOC-1R (deleted in oral cancer 1-related; CDK2AP2) [17], and CDK2 [18]. The interaction of DOC-1 with DNA polymerase alpha/primase, important in cell cycle regulation and initiation of de novo DNA replication, negatively regulates DNA replication at the initiation step and not the elongation phase [16]. DOC-1R/DOC-1 is a substrate of mitogen-activated protein kinase (MAPK) and is important in microtubule association during meiotic maturation [17]. DOC-1 interacts with the free monomeric non-phosphorylated form of CDK2 [18], which regulates the G_1/S phase transition of the cell cycle through complexes with cyclins A and E [19]. DOC-1 inhibits CDK2 kinase activities by sequestering the monomer (inactive) pool of CDK2 by preventing the formation of complexes with cyclins A and E and by targeting CDK2 for the proteasome degradation pathway on overexpression of DOC-1 in cells [18]. Hence, DOC-1 repressed DNA replication by inhibition of DNA polymerase alpha/primase and CDK2 inhibition. Recently, DOC-1 has been shown to play important roles in transforming growth factor beta (TGF-β)-mediated growth suppression independent of $p15^{INK4B}$, and $p12^{CIP1}$ signifies a specific CDK2 inhibitor [20]. Isotope labeling studies revealed that DOC-1 is also a subunit of the Mi-2/NuRD (nucleosome remodeling and histone deacetylase) complex and may be involved in epigenetic gene regulation [21].

The expression of the DOC-1 gene was absent or reduced in the malignant human oral keratinocyte and transformed hamster model [14,15]. DOC-1 is constitutively expressed in normal human tissues, and it interacts with cell cycle regulatory elements [15,16]. The ectopic expression of DOC-1 is associated with decreased cellular CDK2-associated kinase activity in human embryonic kidney 293 cells and it elevates the number of apoptotic cells in the transfected malignant hamster keratinocyte model, implying a possible role for CDK2AP1 in apoptotic pathways [18,22]. These early studies suggested an important role of DOC-1 in carcinogenesis. Recently, several studies have reported negative or decreased DOC-1 expression in gastric cancer tissue, and this was highly correlated with more advanced tumor stage and invasion [23], risk of lymph node metastases, and decreased survival in patients with OSCC [24]. Similar reports were observed from many cancer types including prostate cancer [25], esophageal carcinoma [26], breast cancer [27], colorectal cancer [28], and lung cancer [29].

In the present study, the messenger RNA (mRNA) expression of DOC-1 in patients with OLP, LPK, OSF, and OSCC in comparison to normal control subjects has been investigated and correlated to differential oral habits. In addition, the correlation of DOC-1 mRNA expression with exposure was investigated to determine the role of the DOC-1 gene in the early detection of cancer progression and oral carcinogenesis.

2. Materials and methods

2.1. Patient and control

The clinically and histopathologically confirmed patients with OLP, LPK, OSF, and OSCC were selected from the outpatient department of Dr. R. Ahmed Dental College & Hospital in Kolkata, India. A total of 195 consecutive patients with OLP ($n = 17$), LPK ($n = 42$), OSF ($n = 52$), and OSCC (69) and healthy normal volunteers (controls) ($n = 15$) were recruited to study the mRNA expression of DOC-1 in the biopsy tissue. Detailed oral examination was conducted by a trained clinical oral pathologist. Healthy volunteers without oral habit or oral habit for <6 month with a normal oral epithelium were included. Patients having oral habits for >6 months along with precancerous or cancerous lesions were included in the study. The demographic parameters were recorded using a detailed questionnaire. Patients suffering from infectious/contagious disease, intractable medical or radiological abnormality, other white patches such as in candidiasis, or suffering from scleroderma were excluded from the study. The biopsy specimen was collected from the cheek in the control group and depending upon the location of the lesion in precancer and cancer. In OLP, sites with white striations, white papules, white plaques, erythema, erosions, or blisters affecting predominantly the buccal mucosa were selected. In LPK, sites with white or white-and-red lesions that may be either irregularly or uniformly flat and thin and nodular or exophytic having a wrinkled or corrugated surface with a consistent texture throughout were selected. In OSF, sites with tough, leathery texture of the mucosa, blanching of mucosa (persistent, white, marble-like appearance, which may be localized, diffuse, or reticular), and quid-induced lesion (fine, white, wavy, parallel lines that do not overlap or crisscross, are not elevated, and radiate from a central erythematous area) were selected. In OSCC, sites with red or white, painless, nonhealing, indurated ulcers were selected. The patients with OSCC were descriptively categorized as well-differentiated (grade I), moderately differentiated (grade II), poorly differentiated (grade III), and undifferentiated (grade IV) [30]. The study was approved by an ethical review committee of the institute, and informed consent was obtained from all participating patients and healthy volunteers.

2.2. Sample collection, RNA extraction, and reverse transcriptase polymerase chain reaction

Oral biopsy tissue from the precancerous and cancerous site was washed thrice with sterile 1× phosphate-buffered saline (PBS) and transferred into a 1.5-ml Eppendorf micro-centrifuge tube containing 1 ml of TRIzol reagent (Invitrogen, Grand Island, NY, USA). The sample tubes were stored at −20 °C until further usage. The total RNA was isolated from the whole biopsy tissue specimen (containing both epithelium and connective tissue) using TRIzol (Invitrogen) following the manufacturer's instructions. To the RNA pellet, 40 μl of diethyl pyrocarbonate (DEPC)-treated water was added to ensure complete dissolution of RNA into the solution. A reverse transcriptase polymerase chain reaction (RT-PCR) was performed with 1 μg of RNA using the one-step RT-PCR kit (QIAGEN, Valencia, CA, USA). DOC-1 obtained from the biopsy tissue was amplified using a forward primer, 5′-ATGGCAACGTCTTCACAGTACC-3′, and a reverse primer, 5′-CAGTCCTCTAGCGTGAATGATG-3′. The amplification conditions were as follows: reverse transcription at 50 °C for 30 min, the initial PCR activation step at 95 °C for 15 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 1 min, and the final extension at 72 °C for 10 min. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) used as an internal control for tissue expression was amplified with the forward primer 5′-ATGGGGAAGGTGAAGGTCGG-3′ and the reverse primer 5′-GGATGCTAAGCAGTTGGT-3′. The amplification conditions were as follows: 35 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 1 min. The amplified RT-PCR products were subjected to electrophoresis on 2% agarose gels and stained with ethidium bromide. The gel was placed in a Gel Doc system (PerkinElmer, Waltham, MA, USA) for visualization.

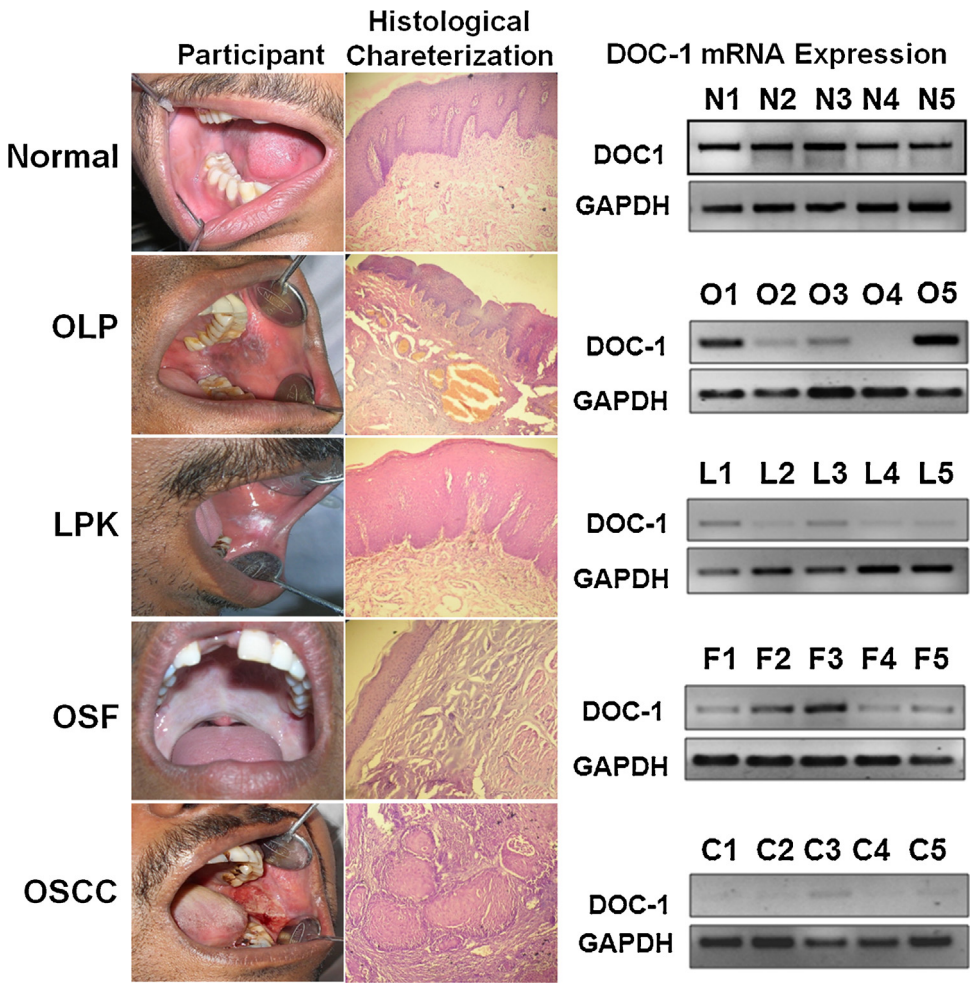

Fig. 1. DOC-1 mRNA expression. The representation of oral precancerous conditions (OLP, LPK, and OSF) and OSCC, the histological characteristics, and DOC-1 mRNA expression in normal control subjects (lane N1–5) and patients with OLP (lane O1–5), LPK (lane L1–5), OSF (lane F1–5), and OSCC (lane C1–5). The expression of GAPDH was used as an internal control for the quantification of DOC-1 mRNA expression.

2.3. Statistical analysis

GraphPad Prism software (San Diego, CA, USA) was used for statistical analysis. The relative intensity of DOC-1 mRNA expression from the image of RT-PCR bands was calculated against the internal control GAPDH using an ImageJ® analysis system (software, National Institutes of Health, Bethesda, MD, USA). A comparison of the expression of DOC-1 mRNA between the OLP, LPK, OSF, and OSCC patient groups and the control group was performed using nonparametric Mann–Whitney tests. A comparison of the expression of DOC-1 between the differential oral habits was performed using nonparametric Mann–Whitney tests. The correlations of DOC-1 mRNA expression with the duration of exposure were analyzed using Spearman's rank test. A p-value <0.05 was set as statistically significant.

Table 1
Demographic characteristics and different oral habit exposures in the study population.

Parameter	Normal	OLP	LPK	OSMF	OSCC
$n = 195$	$n = 15$	$n = 17$	$n = 42$	$n = 52$	$n = 69$
Age (yr)	29.46 ± 7.03	41.35 ± 12.17	47.66 ± 10.91	28.67 ± 9.41	53.87 ± 11.14
Sex					
Female	6 (40.00%)	4 (23.52%)	8 (19.04%)	11 (21.16%)	26 (37.68%)
Male	9 (60.00%)	13 (76.47%)	34 (80.96%)	41 (78.84%)	43 (62.32%)
Exposure (yr)	–	11.35 ± 6.43	19.92 ± 12.43	6.11 ± 5.40	20.23 ± 13.27
Habit					
Areca	–	–	–	40 (76.92%)	7 (10.14%)
Bidi	–	2 (11.76%)	17 (40.4%)	–	16 (23.18%)
Cigarette	–	10 (58.82%)	6 (14.28%)	–	3 (4.35%)
Pan	–	–	8 (19.05%)	12 (23.08%)	19 (27.53%)
Tobacco	–	5 (29.42)	11 (26.19%)	–	24 (34.78%)

Yr; years, Areca; only having habit of areca nut chewing, *Pan*; includes mixture of betel leaf, areca nut, and slaked lime, and may contain tobacco.

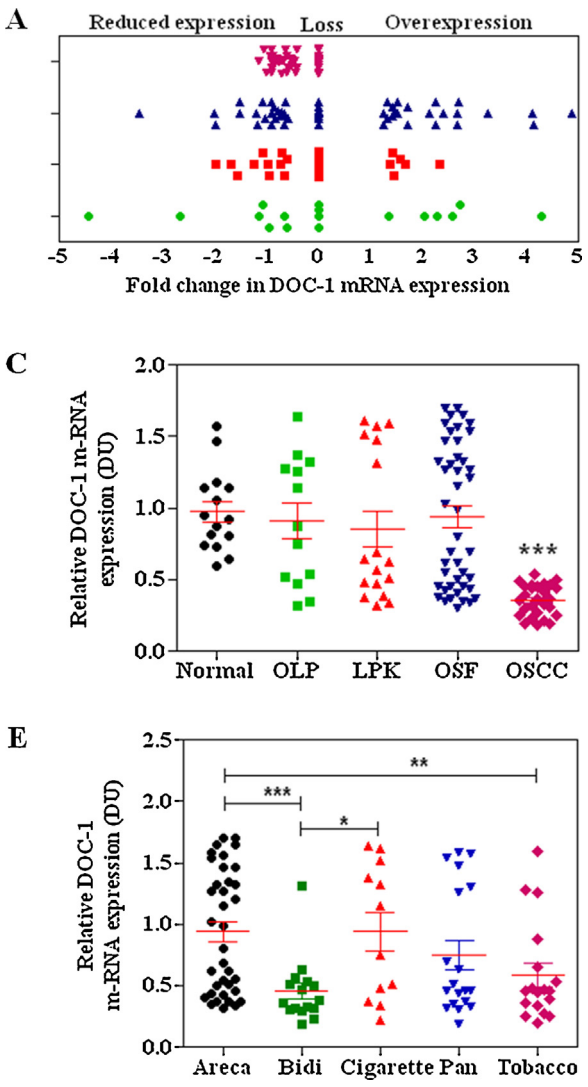

Fig. 2. Quantitative analysis of DOC-1 mRNA expression. (A) The horizontal scattered plot representation of log2 (fold change) in the DOC-1 mRNA expression in precancer (OLP, LPK, and OSF) and OSCC. The non-detectable or loss of expression (zero) and overexpression (positive) and reduced expression (negative) of DOC-1 mRNA was shown. Log2 (relative DOC-1 mRNA expression of case/average of relative DOC-1 mRNA expressions of control). (B) The vertical scattered plot representation of DOC-1 mRNA expression in precancer (OLP, LPK, and OSF) and cancer patients compared to normal. "****" shows the level of significance with *p*-value <0.0001. (C) The vertical scattered plot representation of DOC-1 mRNA expression in differential oral habits (areca, *bidi*, cigarette, *pan*, and tobacco). "*," "**," and "***" shows the level of significance with *p*-value <0.05, <0.001, and <0.0001, respectively.

3. Result

A representative clinical presentation of control subjects and precancer and cancer patients along with DOC-1 mRNA expression is described in Fig. 1. Demographic parameters such as age, sex, and duration of exposure to deleterious oral habits are presented in Table 1. Of the total number of participants in the study, 28.2% were female and 71.8% were male. All participants of the precancer and cancer group had a history of deleterious oral habits with varying durations of exposure. The mean durations of exposure (±standard deviation (SD) in years) of deleterious oral habits among the study patients as observed were as follows: OLP 11.35 ± 6.43, LPK 19.92 ± 12.43, OSF 6.11 ± 5.40, and OSCC 20.23 ± 13.27 (Table 1).

The overexpression and reduced expression of DOC-1 mRNA in the lesion site was calculated as follows: log2 (relative DOC-1 mRNA expression of case/average of relative DOC-1 mRNA expression of control). This results in a positive value for the overexpression and a negative value for the reduced DOC-1 mRNA expression. The non-detectable level of DOC-1 mRNA was designated as a loss of DOC-1 expression (Fig. 2A). The distribution of DOC-1 mRNA expression in the precancer and cancer group compared to the normal control group, among the DOC-1 mRNA-expressing individuals, is shown in Fig. 2B. DOC-1 mRNA expression was detected in all biopsy tissues of the control group ($n = 15$). Interestingly, LPK and OSCC showed a remarkable loss of DOC-1 mRNA expression in ~60% of cases each. Of the total number of cases, 76.41% and 84.61% retained the DOC-1 mRNA expression in the form of either overexpression or reduced expression in OLP (35.29% and 41.17%) and OSF (42.30% and 42.30%), respectively (Table 2). This suggested that the rate of loss of DOC-1 mRNA expression was more frequent in the LPK and OSCC cases than OLP and OSF.

The mean ± SD DOC-1 mRNA expression was significantly reduced (p-value <0.0001) in patients with OSCC (0.357 ± 0.1) compared to control subjects (0.97 ± 0.28) and patients with OLP (0.90 ± 0.44), LPK (0.85 ± 0.51), and OSF (0.94 ± 0.49) (Table 3) (Fig. 2B). However, no significant difference in DOC-1 mRNA expression between healthy control subjects without with oral habits ($n = 15$, 0.91 ± 0.38, $p = 0.49$) was observed (data not shown). The total group of patients could be further stratified into the overexpression or reduced DOC-1 mRNA expression group. The mean ± SD of DOC-1 mRNA expression in overexpressing patients with OLP (1.33 ± 0.16), LPK (1.51 ± 0.0.11), and OSF (1.40 ± 0.21) was significantly higher with p-value being 0.009, 0.002, and <0.0001, respectively, compared to the control group. The mean ± SD of DOC-1 mRNA expression in patients of the reduced expression group with OLP (0.54 ± 0.2), LPK (0.48 ± 0.13), OSF (0.49 ± 0.12), and OSCC (0.35 ± 0.10) was significantly lower with p-value being 0.004, <0.0001, <0.0001, and <0.0001, respectively, compared to the control group.

Moreover, DOC-1 mRNA expression in patients with OSCC was further distributed according to Broders' classification (Table 4).

Table 2
The DOC-1 mRNA expression analysis in precancer and cancer patients compared to normal control subjects.

Clinical condition	*N*	DOC-1 mRNA			
		Expression[b]	Loss	Overexpression	Reduced expression
Normal	15	15 (100.00%)	0 (0.00%)	–	–
OLP	17	13 (76.47%)	4 (23.53%)	6 (35.29%)	7 (41.17%)
LPK	42	17 (40.47%)	25 (59.52%)	6 (14.85%)	11 (26.19%)
OSMF	52	44 (84.61%)	8 (15.38%)	22 (42.30%)	22 (42.30%)
OSCC	69	27 (39.13%)	42 (60.87%)	0 (0.00%)	27 (39.13%)
Sum (Σ)	195	101 (56.11%)[a]	79 (43.89%)[a]	34 (18.89%)[a]	67 (37.22%)[a]

[a] The population of precancer and cancer.
[b] The overall DOC-1 mRNA expression, including both overexpression or reduced expression of DOC-1 mRNA.

Table 3
The mean ± SD DOC-1 m-RNA expression analysis in precancer and cancer patients compared to normal control subjects.

	N	Mean ± SD (DU)	DOC-1 overexpression	DOC-1 reduced expression	*p*-Value[#]
Normal	15	0.9742 ± 0.2864	–	–	<0.0001
OLP	13	0.9083 ± 0.4475	1.334 ± 0.1653[**]	0.5437 ± 0.2045[**]	<0.0001
LPK	17	0.8503 ± 0.5176	1.513 ± 0.0.1100[**]	0.4803 ± 0.1337[***]	<0.0001
OSMF	44	0.9416 ± 0.4981	1.403 ± 0.2105[***]	0.4900 ± 0.1290[***]	<0.0001
OSCC	27	0.3567 ± 0.1069	–	0.3567 ± 0.1069[***]	–

[**] The level of significance having *p*-value <0.001.
[***] The level of significance having *p*-value <0.0001.
[#] The *p*-value obtained after comparing the DOC-1 mRNA expression of OSCC with normal and precancer group using nonparametric Mann–Whitney test.

Table 4
The distribution and DOC-1 mRNA expression analysis in patients with OSCC according to Broders' classification.

Broders' classification	*N*	DOC-1 mRNA expression	
		Loss	Reduced expression
Well differentiated (GI)	55 (79.71%)	28 (40.57%)	27 (39.13%)
Moderately differentiated (GII)	7 (10.14%)	7 (10.14%)	0 (0.00%)
Poorly differentiated (GIII)	6 (8.69%)	6 (8.69%)	0 (0.00%)
Undifferentiated (GIV)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Sum (Σ)	69	42 (60.87%)	27 (39.13%)

Interestingly, in patients with moderately and poorly differentiated OSCC, a complete loss of DOC-1 mRNA expression was observed, whereas patients with well-differentiated OSCC demonstrated a loss of (40.57%) and reduced (39.13%) DOC-1 mRNA expression. This suggested a loss of DOC-1 mRNA expression with progression of OSCC.

The analysis of DOC-1 mRNA expression with respect to oral habits was conducted, and, interestingly, we observed that those with *bidi* smoking and tobacco chewing oral habits showed significantly reduced expression (0.45 ± 0.26 and 0.58 ± 0.4) compared to the control group (*p*-value <0.0001 and <0.05, respectively). Among *bidi*- and tobacco-habituated patients, 45% show significantly reduced DOC-1 mRNA expression and 55% show loss of DOC-1 mRNA expression. The distribution of DOC-1 mRNA expression with different oral habits is shown in Fig. 2C. Similarly, patients habituated to *bidi* show a significantly reduced expression of DOC-1mRNA compared to patients habituated to areca nut (0.94 ± 0.5) and cigarettes (0.94 ± 0.54) (with *p*-values of 0.0004 and 0.0168, respectively) (Table 5). A significant reduction in DOC-1 mRNA expression was observed in tobacco users (0.58 ± 0.4) compared to those who chewed areca (Fig. 2C).

Further, to determine the correlation, if any, between the exposure to deleterious habits and DOC-1 mRNA expression in the precancer and cancer groups, nonparametric Spearman correlation analysis was performed (Fig. 3A–D). A significantly higher negative correlation was observed in OLP ($r = -0.92$, $p < 0.0001$), LPK ($r = -0.79$, $p < 0.0001$), OSF ($r = -0.62$, $p < 0.0001$), and OSCC ($r = -0.44$, $p < 0.0001$) in the reduced DOC-1 mRNA expression group. Overall, we found a highly significant negative correlation ($r = -0.43$, $p < 0.0001$) between the exposure period and DOC-1 mRNA expression (Fig. 3E), but no significant correlation ($r = 0.21$, $p = 0.22$) was found between the overexpression of DOC-1 mRNA and the duration of exposure (Fig. 3F).

Table 5
The mean ± SD DOC-1 m-RNA expression analysis with differential oral habits.

Habit	*N*	Mean ± SD (DU)
Areca	36	0.9395 ± 0.5002
Bidi	16	0.4524 ± 0.2616[***]
Cigarette	12	0.9381 ± 0.5459
Pan	19	0.7474 ± 0.5116
Tobacco	18	0.5859 ± 0.4001[*]
Sum (Σ)	101	

[*] The level of significance having *p*-value <0.05.
[***] The level of significance having *p*-value <0.0001.

4. Discussion

Understanding the molecular mechanism behind the malignant transformation of the oral precancerous condition into OC is critical. The development of therapeutic modalities and identification of an early diagnostic marker is the need of the hour to minimize the malignancy transformation rate. Fundamental studies have unfolded the molecular mechanism behind the molecular control and functional regulation of the cell cycle in the pathogenesis of cancer. However, the critical role of certain proteins, which may have a distinct role in the protection or pathogenesis of cancer and may serve as potentially good biomarkers for early detection, remains to be elucidated. In the present study, we reported the differential mRNA expression of the tumor suppressor deleted in oral cancer-1 (DOC-1) gene, which has not been reported previously in relation to the precancerous conditions of OSF, OLP, and LKP, and differential oral habits. Clinically, a decrease in DOC-1 expression in OSCC and other forms of cancers has been found to correlate with its potential role in malignancy [25–29], whereas in vivo studies have shown that the overexpression of DOC-1 in mouse and hamster model of OC, and HCPC-1 cells significantly induced antitumor response, growth suppression, and an increase in apoptosis and antiproliferative effect [22,31]. In the present study, a loss of DOC-1 mRNA expression was observed in 61% of patients with OSCC and a reduced DOC-1 mRNA expression in 39% of patients. The gradual loss of DOC-1 mRNA expression from the well- to undifferentiated stage of OSCC may have the prognostic and prediction to aggressiveness of lesion.

LPK is considered as the potentially malignant disorder of the oral cavity. Etiological risk factors include cigarette and *bidi* smoking, tobacco chewing, alcohol abuse, vitamin deficiency, and infections [7]. In the present study, nearly 85% of patients with LPK showed either loss of or reduced DOC-1 mRNA expression. A similar decreased expression was also observed in patients with LPK by Wenghoefer et al. [32] and Shintani et al. [33]. However, a similar pattern of DOC-1 mRNA expression was observed in LPK as that of OSCC. Hence, the higher malignancy transformation rate of LPK into OSCC can be further correlated in terms of DOC-1 mRNA expression.

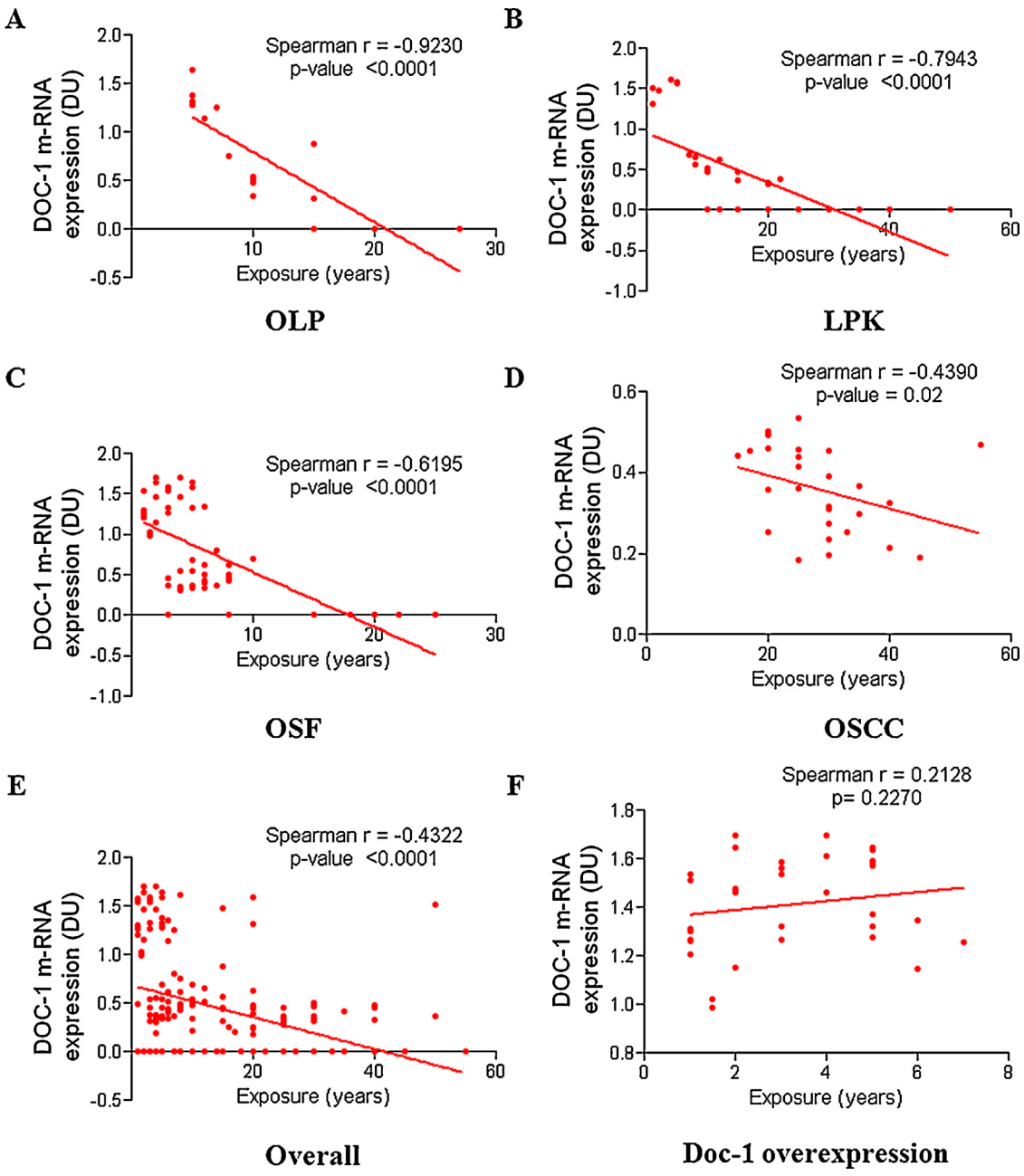

Fig. 3. Nonparametric Spearman correlation analysis. Correlation between the DOC-1 mRNA expression in tissue and exposure to deleterious oral habits of patients with OLP (A), LPK (B), OSF (C), OSCC (D), overall (E), and only DOC-1 overexpression (F). The Spearman correlation coefficient (r) and p-value were indicated.

OSF is a chronic premalignant disease of the oral mucosa associated with the areca nut-chewing habit. This condition is characterized by inflammation and progressive accumulation of collagen fibers in the lamina propria and deeper connective tissue followed by stiffening of the mucosa resulting in difficulty in mouth opening called "trismus" [34]. The epidemiological evidence suggests that chewing of areca nut is the major risk factor for OSF development [8,10], but not all chewers developed OSF. This implies that there must be certain factors such as genetic predisposition or epigenetics that may explain such individual variability. In the present study, areca nut chewers showed unregulated and downregulated distribution of DOC-1 mRNA expression. This suggested that there must be certain other factors along with oral habits playing a major role in the modulation of DOC-1 expression. The overexpression of DOC-1 mRNA in the case of OSF could be correlated with its protective action or it is delayed in the malignant transformation of OSF into OSCC, and it may act as an indicative marker.

OLP is a mucocutaneous disease due to nonspecific inflammation leading to intense destruction of the basal layer of the epithelium [35]. It has often been questioned whether patients with OLP have a hereditary predilection to malignant transformations. However, the risk of malignant transformation in OLP is lower than in LPK and OSF. Smoking has been considered to have the highest prevalence in initiating OLP, also associated with tobacco and areca nut chewing [35]. In the present study, overexpression of DOC-1 mRNA was observed in 35% of patients with OLP. This could be explained as the protective response toward the stressed induced in the mucosa of OLP due to oral habits.

Attempts were also made to correlate DOC-1 mRNA expression with the duration of exposure to deleterious oral habits. A significantly negative correlation of OLP, LPK, and OSF with respect

to an increase in the duration of exposure especially in patients using tobacco (smoking/smokeless) indicates the effect of deleterious oral habits on the development of oral carcinogenesis. Notably in the initial stage of exposure to oral habits, DOC-1 mRNA expression was upregulated in certain precancer patients, which has been observed to gradually decrease in patients having more than 10 years of oral habit. These observations may emphasize the tumor-suppressing action of DOC-1 or the protective role of DOC-1 against the oncogenic stimulus at the initial stages, which has also been demonstrated in several other in vitro studies [13,22].

Winter et al. have shown the reduction of DOC-1 mRNA expression in the biopsy tissue of irritation fibromas (0.7), LPK (0.2), and OSCC (not traceable) compared to healthy gingiva (baseline 1) and its expression by immunohistochemistry (IHC) study on the same sample [36]. This study further suggested the tight regulation between DOC-1 mRNA and protein expression as shown by IHC. Moreover, the RT-PCR is the most sensitive method of mRNA detection even at small amounts of mRNA. In the early diagnosis of precancerous conditions or to detect the aggressive nature of cancer, the RT-PCR method has frequently been used. Therefore, the present study has been limited to analysis of DOC-1 mRNA expression using RT-PCR analysis, and it warrants further study at the translational level along with additional biomarkers to make DOC-1 a better indicative marker for oral precancer and OSCC.

To conclude, we believe our observation of the differential expression of DOC-1 mRNA in patients with OLP, LPK, OSF, and OSCC in comparison to controls is significant given to its established role in growth control, cell cycle kinetics, and DNA replication [15,16,18,20]. We anticipate that a further study regarding the role of DOC-1 in cell cycle regulation and apoptosis may partially explain the fine mechanism between the premalignant and malignant state of a cell. Similarly, the parameter or the molecular event that controls the upregulation and downregulation of DOC-1 expression response to stress is still elusive; therefore, further research is required. The present study further proposes the DOC-1 as a promising marker at least to differentiate the state of a premalignant tissue and further assists in developing novel prevention and treatment strategies.

Conflict of interest

None declared.

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