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Original Article

Correlated analysis of semi-quantitative immunohistochemical features of E-cadherin, VEGF and CD105 in assessing malignant potentiality of oral submucous fibrosis

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ABSTRACT

Oral submucous fibrosis, a potentially premalignant condition for oral squamous cell carcinoma, manifests both non-dysplastic and dysplastic grades. Early and specific identification of its malignant potentiality suffers from diagnostic limitations that may be addressed by correlated molecular pathology attributes having histopathological backdrop. Present study correlates expressional alteration in prime epithelial marker E-cadherin, with neo-angiogenic molecules *viz.* VEGF and CD105 for elucidation of malignant potentiality in different stages of oral submucous fibrosis. Sixty-eight incision biopsies from normal oral mucosa ($n = 10$), non-dysplastic ($n = 18$) and different dysplastic grades ($n = 40$) of oral submucous fibrosis were semi-quantitatively analyzed for immunohistochemical expressions of E-cadherin (membranous and cytoplasmic), VEGF and CD105 which were further statistically correlated. The loss of membranous E-cadherin with increase in cytoplasmic accumulation in differentiative layers of epithelium through the progression of dysplasia was noted along with up-regulation in VEGF expressions. The number of $CD105^+$ blood vessels and their major axis also showed significant increase from non-dysplasia toward higher grades of dysplasia. The positive correlation between deregulated expression of epithelial cell–cell adhesion molecule and increase in neo-angiogenic attributes of oral submucous fibrosis with increase in dysplastic grades indicated elucidatory potential of molecular expression features in assessment of malignant potentiality in oral submucous fibrosis.

Introduction

Globally, oral cancer is a major malignancy with mortality rate of 30% in males and 12% in females [20]. Despite extensive research, 5 years survival rate of oral cancer patients could not be extended. It is now understood that effective addressing of this problem requires critical attention to the domain knowledge inadequacy for early and specific diagnosis of malignant potentiality in premalignant lesions/conditions [24]. In this regard, semi-quantitative and quantitative analysis of pathobiological attributes especially in considering vital molecular pathology features related to alteration in functional integrity and cancer suppressive/inductive properties of oral mucosa and its epithelium in particular may be contributory [22].

It is reported that oral cancer arises from multifocal areas of oral mucosa with high malignant potentiality and these are known as potentiality premalignant lesions [48]. Oral submucous fibrosis (OSF) is a potentially premalignant condition having high prevalence (0.3–3.2%) in South-Asian population with malignant transformation rate of 7–13% [4,30,49]. Clinically, patients with OSF exhibit marked rigidity of oral mucosa and inability to open mouth (trismus). Histopathologically, it is characterized with slow progressive fibrosis of lamina propria, atrophic epithelium with/without dysplasia and chronic inflammation [36,46]. Most common etiological factor of OSF is tobacco and areca nut chewing with betel quid [46]. It has been opined that fibrosis in OSF is associated with altered collagen metabolism and reduced vascularity under the assaults of carcinogenic agents present in areca nut [21]. In OSF, different grades of epithelial dysplasia are

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contributory to its high malignant transformation rate (7–13%) [4,43]. Dysplasia not only jeopardizes oral epithelial integrity but also affects the process of progressive maturation [43]. During the process of progressive maturation in oral epithelium, proliferative cells from lower layers (proliferative layer of epithelium) enter the maturating compartments in upper layers (differentiative layer of oral epithelium) where they undergo differentiation with concomitant modulation in relevant cellular processes [44]. However, with development of dysplasia, this epithelial compartmentalization being effected shows cytological and architectural changes. Hence, improvising knowledge base on related molecular pathology features in two distinguished compartments of oral epithelium through semi-quantitative/quantitative analysis could be vital to elucidate malignant potentiality of this pathosis.

The proliferative zone of normal oral epithelium essentially includes biological attributes for cellular migration [44] which is depicted by the predominance of cytoplasmic E-cadherin whereas differentiative layers have a prominent expression of membranous form. E-cadherin is a prime calcium dependent epithelial glycoprotein responsible for cell–cell adhesion, molecular signaling in sustaining epithelial integrity and the maturation dynamics of epithelial tissue [37,41]. In fact extracellular domain of this adhesion molecule establishes contact with neighboring cells to form adherens junction whereas its cytoplasmic domain interacts with actin cytoskeleton *via* β- and α-catenins for regulation of cell signaling and cellular migration [37]. During pre-malignant and malignant changes in stratified epithelium a deregulation occurs in proteomic forms of E-cadherin [11] alluding the plausibility of migratory phenotype development [1,9,23].

Epithelial dysplasia is well known to be associated with abnormal cell proliferation, cellular atypia and loss of intercellular adhesion along with appearance of migratory features [43]. These pathobiological attributes concurrently experience hypoxia in epithelium which itself acts as an angiogenin and can trigger subepithelial angiogenesis [45]. This subepithelial angiogenesis plays critical role in metastasis of premalignant conditions [29]. In case of epithelial dysplasia of oral premalignant conditions/lesions such as leukoplakia and oral lichen planus, there is no report on change in microvessel density in non-dysplastic epithelium [31]. However, in dysplasia, the subepithelium showed an elevation in microvascular density [3,14]. Further, it may be noted that the avascular nature of fibrotic sub-epithelium in OSF may act as an obstruction for angiogenesis unlike in other premalignant lesions [14,32]. Therefore, understanding the modulation in angiogenic regulator might help in providing information on malignant potentiality of OSF. In this regard, vascular endothelial growth factor (VEGF), a critical regulator of both physiological and pathological angiogenesis [39] need to be understood both in terms of intensity of expression and spatial distribution in tissue. The increased expressions of VEGF in oral squamous cell carcinoma (OSCC) [3,8,18,25,27,42] and in potentially premalignant conditions/lesions have been reported by various researchers [3,8,14,18,27]. However, considering the malignant potentiality of OSF, analysis of vasculature showing CD105 (endoglin) positivity is also important [12] as this molecule expresses most abundantly in actively growing endothelial cells of tumor associated vasculature rather than normal [13]. Thus a correlated analysis of these molecular expression features (semi-quantitative/quantitative) at the immunohistochemical levels could be of significant contribution to molecular pathology to assess malignant potentially in OSF.

In this study a correlated analysis on semi-quantitative immunohistochemical features (intensity and spatial distribution) of E-cadherin at proliferative and differentiative layers in order to recognize oral epithelial integrity and cancer suppressive status along with VEGF and CD105, in the context of neo-angiogenesis at oral subepithelium has been adopted to elucidate vital molecular pathology attributes in assessing malignant potentiality and progression of OSF stages.

Table 1
Clinicopathological conditions of the study subjects.

Feature	No. of patients	Percentage
Incision biopsies	68	
Oral mucosal condition		
Normal (NOR)	10	14.70
OSF	58	85.29
Without dysplasia (OSFWT)	18	26.47
With dysplasia	40	58.82
Mild dysplasia (OSFWM)	20	29.41
Moderate dysplasia (OSFWMO)	10	14.7
Severe dysplasia (OSFWS)	10	14.7
Gender		
Male	43	63.23
Female	25	36.76
Age (years)		
<40 years	48	70.5
Male	28	41.17
Female	20	29.41
>40 years	20	29.41
Male	15	22.02
Female	5	7.35

Materials and methods

Inclusion of study samples

Present study was conducted on 68 incision biopsies from buccal mucosa (Table 1) comprising 10 normal (NOR) and 58 oral submucous fibrosis (OSF) [18 without dysplasia (OSFWT) and 40 with dysplasia (OSFWD)] samples. Furthermore, the OSFWD samples were classified (Table 1) as OSF with mild dysplasia (OSFWM), OSF with moderate dysplasia (OSFWMO), and OSF with severe dysplasia (OSFWS). The exclusion–inclusion criteria applied for choosing patients/samples are as follows: all the patients considered for the study had a deleterious habit of chewing areca nut, panmasalaa, *etc.* with characteristic symptoms of OSF; all the samples were histopathologically confirmed as OSF by oncopathologists and showed no co-morbidity with other oral precancers. The OSF biopsies were collected from buccal mucosa. Normal specimens were collected form disto-buccal aspect of the third molar teeth. All the selected individuals were suffering from impacted mandibular third molar teeth and were in need of trans-alveolar extraction. The excess amount of muco-periosteal buccal flaps that has been left after trans alveolar surgeries have been excised and used as normal sample. All the samples were collected from Guru Nanak Institute of Dental Science and Research (GNIDSR), Kolkata, after informed consent from patients and ethical clearance of GNIDSR (ethical clearance Number GNIDSR/IEC/07/16 dated 20/11/07) which was in full accordance with ethical principles and guidelines of Indian Medical Association, including the World Medical Association as per Helsinki declaration. The patients had undergone biopsy during the period of 2008–2013.

Tissue processing

The biopsy specimens were fixed in phosphate buffered formalin and embedded in paraffin. 4 μm thickness tissue sections were collected on poly-L-lysine coated glass slide. For immunohistochemical studies, sections were baked, de-waxed, re-hydrated using graded alcohol and heated in microwave in Tris EDTA buffer for 20 min using EZ-antigen Retrieval System V2 (BioGenex, Sen Ramon, CA, USA). Sections were immunostained with following monoclonal antibodies: anti-E-cadherin, (clone EP700Y, Cat. No. ab40772, Abcam, Cambridge, UK), anti-CD105 (clone 4G11,