Original Article

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Dentistry Section

Assessment of Malignant Potentiality of Oral Submucous Fibrosis Through Histomorphometry and E-cadherin Expression: A Cross-sectional Study

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ABSTRACT

Introduction: Oral Submucous Fibrosis (OSF) is an oral precancerous condition with highest potential for malignant transformation. When OSF advances to oral cancer, epithelial cells undergo several dysplastic changes that alter the epithelial properties and architecture. Analysis of these features can provide useful diagnostic information to assess malignant potentiality of the disease process to avoid progression to oral cancer.

Aim: The aim of the study was to assess the progression of non dysplastic and dysplastic OSF to malignancy using histomorphometry and E-cadherin expression in different layers of epithelium.

Materials and Methods: The present cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research (GNIDSR), Panihati, Kolkata, and School of Medical Science and Technology (SMST), IIT, Kharagpur, West Bengal, India during the period of December 2019 to August 2021. It included 50 subjects divided into two groups, with 43 individuals suffering from OSF and seven individuals without disease process. Biopsy was conducted to establish diagnosis of OSF and stained sections were classified into nondysplastic and dysplastic category. Some sections were also prepared on lysine coated slides for immunohistochemical analysis. Finally, both sections were taken to SMST, IIT, Kharagpur, India, for staining with E-cadherin antibody and for procurement of photomicrographs using inverted microscope to undergo histomorphometrical analysis in basal-parabasal layers of epithelium and for assessing/comparing expression of adhesion molecule (E-cadherin) in basal-parabasal and spinous layers of non dysplastic and dysplastic OSF tissue using Image J software.

Results: The mean cell area was found to be gradually increased from Normal Oral Mucosa (NOM) (35.60±3.26) to OSF with dysplastic (wd (39.12±4.99) followed by OSF without dysplastic (wtd) (64.11±9.90). The mean value of major axis was highest in OSFwtd (14.53±3.20) in comparison to OSFwd (11.23±1.98) and NOM (8.14±0.99), whereas the mean of minor axis was found to be decreased in OSFwd (4.24±0.89) and increased in case of OSFwtd (6.62±1.11) when compared with NOM (6.60±0.83). aspect ratio was highest in OSFwd (2.82±0.86), which showed gradual decrease in OSFwtd (2.37±0.71) and NOM (1.24±0.08). The gray scale value of membranous expression of E-cadherin in basal-parabasal layers was found to be highest in OSFwd (124.6±14.8) whereas it showed decrease in cytoplasmic expression in OSFwd (89.85±20.08) indicating loss of E-cadherin expression in cell membrane and simultaneous accumulation in cell cytoplasm (as gray scale value is inversely proportional to E-cadherin expression).The spinous cell layer also showed increased membranous gray scale value in OSFwd (143.17±15.5) and decreased cytoplasmic gray scale value in OSFwd (93.03±6.98).

Conclusion: The study concluded that semiquantitative light microscopic histomorphometrical parameters like cellular size (area, major axis, minor axis) and shape (aspect ratio) depicting various statistically significant alterations, along with membranous loss of E-cadherin with concomitant cytoplasmic accumulation, both in basal-parabasal and spinous layers of the surface epithelium can be regarded as a significant indicator in predicting the disease progression of OSF to malignancy.

Keywords: Dysplasia Gray scale, Malignant transformation, Precancerous condition

INTRODUCTION

Oral Submucous Fibrosis (OSF) is a chronic, progressive, scarring disease that mostly affects the people of South-East Asia. Schwartz, first described it as 'Atrophia Idiopathica (Tropica) Mucosae Oris' in 1952 and later, Joshi (1953), renamed it 'OSF' hinting mainly its histological nature [1] whereas, Passi D et al., described it as an oral precancerous condition [2]. Recent surveys (2017) indicate a rise in OSF prevalence in India from 0.03 to 6.42% [3]. OSF is most frequently observed in age groups ranging between 25 to 35 years (2^{nd}-4^{th} decade) with male-female ratio varying by geographical area, but females tend to predominate [2-4]. The commonly affected sites are the buccal mucosa, retromolar region and soft palate. Early cases of OSF manifests with burning sensation, blister formation particularly on the palate, ulcerations, recurrent generalised oral mucosal inflammation, hyper salivation, impaired gustatory sensation and dry mouth [1]. Sub mucosal fibrosis is the hallmark of the disease affecting most of the oral cavity, pharynx and upper third of the oesophagus causing dysphagia and progressive trismus [3]. The oral mucosa becomes blanched and slightly opaque, with appearance of white fibrous bands as the condition advances [1]. Areca nut is the principal aetiological factor of OSF, although, other causative agents may include lime, tobacco, chillies, nutritional deficiencies, immunological and collagen diseases [3]. The characteristic histopathological features include atrophic or hypertrophic surface epithelium with flattening of rete ridges, dense sub epithelial deposition of collagen fibres with different grades of hyalinisation and homogenisation, perivascular fibrosis, chronic inflammatory cell infiltration associated with varied

degrees of epithelial dysplasia (10-15%) [5]. OSF is now worldwide acknowledged as disease of Indian subcontinent with highest risk for malignant transformation, ranging between 7-30% compared to other premalignant oral lesions [3,6].

Though, histopathology is the gold standard for diagnosis of this condition, but researchers are unable to assess its malignant transformation. Recently, in this respect cell to cell adhesion has received much attention about how these cell to cell and cell to matrix interactions forming specialised junctions coordinates to affect the earliest progression of neoplasia [7]. Tight Junctions hold the opposing cell membranes in close proximation with help from transmembrane adhesive molecules arranged in anastomosing strands encircling the cell and include polarity-related proteins playing a crucial role like a "fence" to maintain the two major domains, the apical and basolateral surfaces of the cell membrane [8]. Adhesive junctions anchor cells to the extracellular matrix. The principal transmembrane proteins are cadherins and catenins and others may include integrin, nectin, p120 catenin, afadin, vinculin and ponsin. cadherins are calcium ion-dependent complexes interacting homotypically with cadherins on the adjacent cell. Catenins interact with cytoplasmic domain of the transmembrane cadherin molecule [8]. The malignant epithelial neoplasms are associated with aberrant expression of these molecules leading to disrupted cell to cell and cell to matrix adhesion which has been commonly noted in Oral Squamous Cell Carcinoma (OSCC) [8-11]. Khurseed M et al., suggested that these cell junctions and associated proteins also maintains the apicobasal polarity, the cardinal molecular feature of adult eukaryotic epithelial cells and helps in functions like polarised cell migration and tissue structure maintenance. Loss of cell polarity is a trademark of carcinoma, and several studies indicated that cadherin-mediated cell to cell adhesion are frequently altered in tumour cells. E-cadherin is frequently lost in advanced stages of epithelial cancer progression [7,12-15].

Therefore, the present research was designed with the aim to record the variations in the histomorphometrical parameters along with the gradual changes in expression of E-cadherin in basal-parabasal and spinous layers of surface epithelium to assess the progression of OSF towards malignancy.

MATERIALS AND METHODS

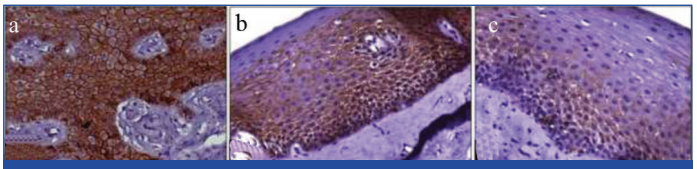
The present cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research (GNIDSR), Panihati, Kolkata, and School of Medical Science & Technology (SMST), IIT, Kharagpur, West Bengal, India, during the period of December 2019 to August 2021. The entire study was accorded ethical clearance from GNIDSR (GNIDSR/IEC/19-22/18).

Initially, 62 patients having OSF along with 10 healthy individuals were selected from the Outpatient Department for the study based on the clinical guidelines by Neville W et al., [5].

Inclusion and Exclusion criteria: Individuals above 18 years of age with the disease process were included in the study whereas subjects suffering from any systemic disorders were excluded. Finally, after detailed clinical examination, systemic investigations and obtaining informed consent for further procedures, forty-three patients and seven normal individuals were selected. Clinical photographs were taken from appropriate sites.

Study Procedure

Incisional biopsy was performed from representative site followed by tissue fixation in 10% Neutral Buffered Formalin (NBF), processing and then staining using Haematoxylin (H) and Eosin (E) as stated by Suvarna KS et al., [16]. All Haematoxylin & Eosin (H &E) stained sections were viewed and evaluated using light microscope with objectives 10x (100x magnification), 20x (200x magnification) and 40x (400x magnification) and diagnosis of NOM and OSF were established. The OSF tissues were further grouped into non dysplastic (OSFwtd) and dysplastic (OSFwd). After this, selections were made from NOM, OSFwtd and OSFwd tissues for further Immunohistochemical staining with E-cadherin marker [Table/Fig-1]

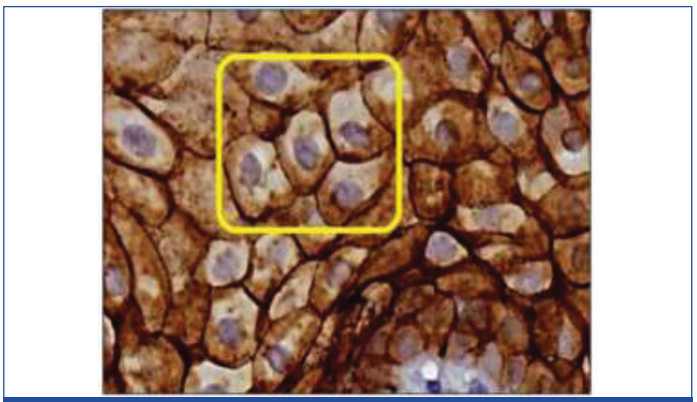


[Table/Fig-1]: Photomicrograph showing E-cadherin stained sections (20x) of: (a) Normal Oral Mucosa (NOM); (b) Oral Submucous Fibrosis (OSF) without dysplasia (OSFwtd); (c) Oral Submucous Fibrosis (OSF) with dysplasia (OSFwd).

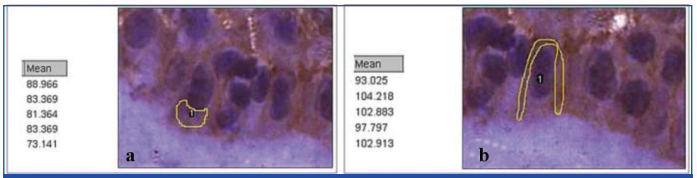
in SMST, IIT, Kharagpur, West Bengal, India, where those slides were viewed under inverted microscope for assessing expression of adhesive molecules.

From each H & E stained sections, Region Of Interest (ROI) was selected consisting of an average of 5 basal-suprabasal cells adjacently. Sixty such ROI's were taken from NOM, dysplastic epithelium in OSF (areas with cellular and nuclear alteration and pleomorphism) and non dysplastic OSF which were subjected to semiquantitative histomorphometric analysis with objectives of 40x (400x magnification) using the freehand selection tool of Image J software. The cellular parameters were area, length of major axis, length of minor axis, aspect ratio (major axis/minor axis).

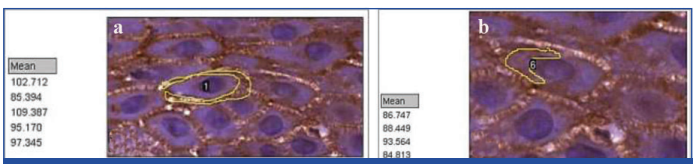
Similar fields, like H&E were also selected from Immunohistochemistry (IHC) stained sections and one box consisting of an average of five cells from both basal-parabasal and spinous layer of epithelium was selected. Ten such boxes were taken from NOM, OSFwd and OSFwtd in both the layers of epithelium under 20x (200x magnification) [Table/Fig-2]. By using the Image J software, the molecular expression of membranous and cytoplasmic E-cadherin of both layers in terms of mean gray scale value were evaluated [Table/Fig-3,4].



[Table/Fig-2]: Photomicrograph showing selection of Region of Interest (ROI) in E-cadherin stained sections (40x).



[Table/Fig-3]: Photomicrograph showing measuring of gray scale values (inversely proportional to E-Cadherin staining intensity) at (a) cytoplasm and (b) membrane of basal cell layer using image J software (20x).



[Table/Fig-4]: Photomicrograph showing measuring of gray scale values (inversely proportional to E-Cadherin staining intensity) at (a) Membrane and (b) Cytoplasm of spinous cell layer using image J software (20x).

STATISTICAL ANALYSIS

Both descriptive and probabilistic statistics were employed. In descriptive statistics, primarily the data was plotted in a bar diagram. Thereafter, Whisker's wide box plot was formulated and drawn to show the distribution of the data in terms of minimum-maximum and percentile distribution. Thereafter, to understand the statistical significance unpaired student t-test was performed (MATLAB; Version 2017a).

RESULTS

On analysing clinicoepidemiological parameters, a considerable number of patients were in age range between 20-30 years 15 (35%) followed by 13 (30%) in 31-40 years. A 29 (67%) amongst OSF patients were males, while 14 (33%) were females. Normal individuals from both gender Male=4, Female=3 within the age range of 18-35 years. Semi quantitative analysis was performed on digitally stored images of H & E stained sections from NOM and OSF in respect to various parameters involving basal and para-basal cells like cellular area, aspect ratio, length of major and minor axis and data was plotted in whiskers box plot.

On evaluating mean cell area, it was found to be more increased in OSFwtd (64.11±9.90) in comparison to both OSFwd (39.12±4.99) and NOM (35.60±3.26) and was statistically significant in all conditions (p-value <0.05) [Table/Fig-5a,6]. After analysing mean length of major axis, it was found to be 8.14±0.99 in NOM, which showed gradual increase in OSFwd (11.23±1.98) followed by OSFwtd. An overall significance was observed between all the conditions (p-value <0.05) [Table/Fig-5b,6]. On comparing mean length of minor axis, it considerably decreased in OSFwd (4.24±0.89) and increased in OSFwtd (6.62±1.11) in comparison to NOM (6.60±0.83). On comparing the 'p' values, it was significant between NOM vs. OSFwd and OSFwtd vs. OSFwd only (p-value <0.05) [Table/Fig-5c,6]. Mean aspect ratio was found to be 1.24±0.08 in NOM with significant increase (p<0.05) in OSFwtd (2.37±0.71) which further more increased in OSFwd (2.82±0.86) [Table/Fig-5d,6].

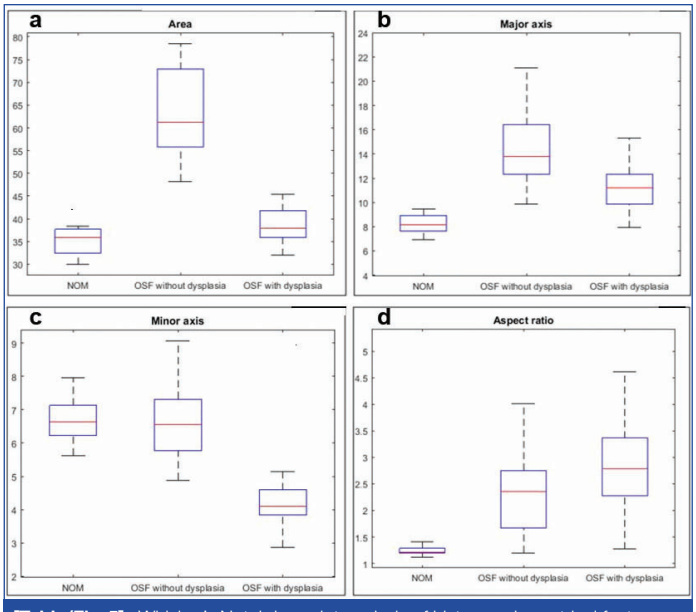
E-cadherin expressions were obtained by measuring mean gray scale values (inversely proportional to E-cadherin staining intensity) of cell membrane and cytoplasm in both basal-suprabasal and spinous cell layers of surface epithelium with the support of image J software. The mean gray scale values of E-cadherin expression in cell membrane of basal-parabasal layer, demonstrated significant increase from 23.20±6.5 in NOM to 109±11.93 in OSFwtd to 124.6±14.8 in OSFwd. Thus, the staining intensity significantly decreased from NOM to OSFwtd to OSFwd. On comparing the mean values, it was found to be significant (p<0.05) between all the pathophysiological conditions [Table/Fig-7a,8]. Evaluation of mean gray scale values of membranous E-cadherin expression in cells of spinous layer, it showed significant increase from 46.41±12.74 in NOM to 108.55±22.01 in OSFwtd to 143.17±15.5 in OSFwd. Thus, the intensity of expression significantly decreased from NOM to OSFwtd to OSFwd. Comparison of the mean values showed statistical significance (p-value <0.05) between all [Table/Fig-7b,8].

Assessment of the mean gray scale values in cell cytoplasm of basal-parabasal layer, they were found to be significantly decreased from 103.24±26.14 in NOM to 97.1±10.38 in OSFwtd to 89.85±20.08 in OSFwd. Therefore, the intensity increased from NOM to OSFwtd to OSFwd. Statistical significance were obtained between only NOM vs. OSFwd and OSFwd vs. OSFwtd (p-value <0.05) [Table/Fig-7c,8].

On assessing mean gray scale values of cytoplasmic expression in spinous layer, it showed significant decrease from 131.79±13.9 in NOM to 126.17±8.14 in OSFwtd to 93.03±6.98 in OSFwd. Therefore, the intensity significantly increased from NOM to OSFwtd to OSFwd. The mean values were found to be significant (p-value <0.05) between all pathophysiological conditions [Table/Fig-7d,8].

Histomorphometrical parameters	Mean values recorded	Conditions	p-value	Statistical significance (p<0.05)
Cell area (μm²) (Mean±SD)	NOM: 35.60±3.26	NOM vs. OSFwtd	2.035E-17*	Significant
	OSFwtd: 64.11±9.90	NOM vs. OSFwd	0.013	
	OSFwd: 39.12±4.99	OSFwtd vs. OSFwd	1.121E-16	
Major axis (Mean±SD)	NOM: 8.14±0.99	NOM vs. OSFwtd	9.067E-20	Significant
	OSFwtd: 14.53±3.20	NOM vs. OSFwd	9.378E-11	
	OSFwd: 11.23±1.98	OSFwtd vs. OSFwd	6.636E-08	
Minor axis (Mean±SD)	NOM: 6.60±0.83	NOM vs. OSFwtd	0.918	Not Significant
	OSFwtd: 6.62±1.11	NOM vs. OSFwd	1.384E-14	Significant
	OSFwd: 4.24±0.89	OSFwtd vs. OSFwd	6.685E-18	
aspect ratio (Mean±SD)	NOM: 1.24±0.08	NOM vs. OSFwtd	1.494E-15	Significant
	OSFwtd: 2.37±0.71	NOM vs. OSFwd	2.093E-13	
	OSFwd: 2.82±0.86	OSFwtd vs. OSFwd	0.009	

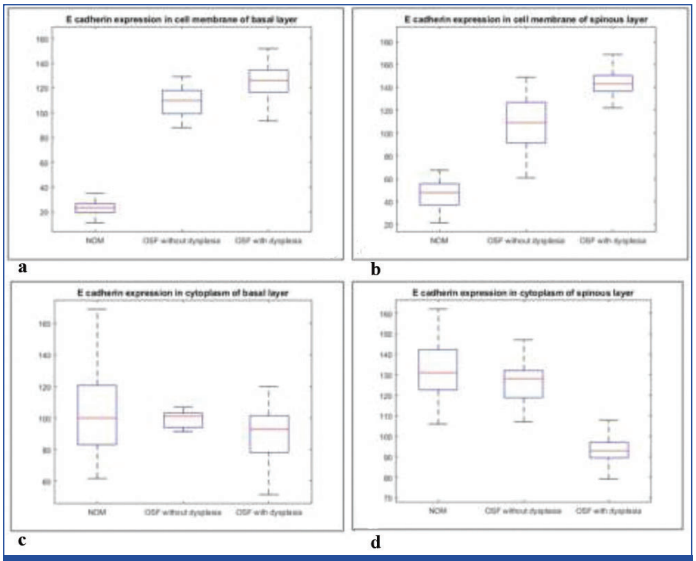
[Table/Fig-6]: Histomorphometrical findings of different pathophysiological conditions with statistical significance (p-value <0.05).
NB (*In most popular programming languages, 2.035E-17 (or 2.035e17) is equivalent to 2.035×1017, and 2.035×10-17 would be written 2.035E-17)



[Table/Fig-5]: Whisker's Notch box plot analysis of histomorphometrical features among different study groups (NOM, OSFwtd and OSFwd): (a) Cell area; (b) Major axis; (c) Minor axis; (d) aspect ratio.

DISCUSSION

The OSF is a common premalignant condition marked by a progressive fibrosis of the lamina propria and deeper connective tissue. It is prevalent around the globe but mainly found in Indian subcontinent. It may show surface epithelial dysplasia (10-15%) with malignant transformation rate 7-30% [5,6]. It can result in OSCC, a risk which is further increased by concomitant tobacco consumption. The present study analysed clinicoepidemiological parameters of OSF (Dysplastic and non dysplastic) like age and sex. The current study recorded majority of cases in the age group of 20-30 years (35%) followed by 31-40 years (30%). Several studies [17,18] also reported OSF cases predominantly in between 20-40 years, which were in accordance to the present study. Previously,



[Table/Fig-7]: Whisker's Notch box plot analysis of E-Cadherin expression in different pathophysiological conditions in cell membrane of (a) Basal and para-basal layers (b) Spinous celllayers; and in cell cytoplasm of (c) Basal and para-basal layers (d) Spinous cell layers.

Shabana AH et al., (1987) performed morphometric analysis of basal cells in oral premalignant lesions and OSCC and noted increase in basal cellular area in lesions associated with malignant changes [24]. Gao S et al., (1995) noticed morphometric parameters of OSF between normal epithelium and dysplasia suggesting it to be an intermediate lesion with biological behaviour and aggressiveness in between normal epithelium and dysplasia [25]. These studies were in accordance with the result of the present study.

Thus, it can be opined that parameters like cell area, major and minor axis can be regarded as an important indicator for progression of disease process for NOM to OSF. Histopathological evaluation forms the backbone in the diagnosis of premalignant conditions like OSF which is further enhanced by immunohistochemical assessment. In this regard, molecular marker like E-cadherin has received phenomenal attention. E-cadherin is one of the most important molecules in cell to cell adhesion that is generally localised to lateral surface of epithelial cells in the adherens junction. As member of a large family of genes coding for calcium-dependent cell adhesion molecules, these glycoproteins are expressed by variety of tissues, mediating adhesion through homotypic binding [26,27]. Therefore, reduced E-cadherin level causes mesenchymal cells to detach from each other and from neighbouring epithelial cells, resulting in loss of coordination between them and this variation of expression has been frequently correlated with various oral precancerous lesions and conditions, and the tumour differentiation of oral cancers [28].

Cell layers	Membranous/cytoplasmic	Mean values recorded	Conditions	p-value	Statistical significance (p<0.05)
Basal and supra-basal cell layer	Membranous expression (mean±SD)	NOM: 23.20±6.5	NOM vs. OSFwtd	2.759E-26	Significant
		OSFwtd: 109±11.93	NOM vs. OSFwd	2.185E-50	
		OSFwd: 124.6±14.8	OSFwtd vs. OSFwd	1.524E-05	
	Cytoplasmic expression (mean±SD)	NOM: 103.24±26.14	NOM vs. OSFwtd	0.169	Not Significant
		OSFwtd: 97.1±10.38	NOM vs. OSFwd	0.006	Significant
		OSFwd: 89.85±20.08	OSFwtd vs. OSFwd	0.045	
Spinous cell layer	Membranous expression (mean±SD)	NOM: 46.41±12.74	NOM vs. OSFwtd	1.954E-28	Significant
		OSFwtd:108.55±22.01	NOM vs. OSFwd	5.433E-54	
		OSFwd: 143.17±15.5	OSFwtd vs. OSFwd	3.097E-14	
	Cytoplasmic expression (mean±SD)	NOM: 131.79±13.9	NOM vs. OSFwtd	0.0167	Significant
		OSFwtd: 126.17±8.14	NOM vs. OSFwd	1.083E-27	
		OSFwd: 93.03±6.98	OSFwtd vs. OSFwd	1.850E-38	

[Table/Fig-8]: Immunohistochemical analysis of Gray scale value (inversely proportional to E-Cadherin staining intensity) of different pathophysiological conditions with statistical significance (p-value <0.05).

Seedat HA and van Wyk C 1988 reported female predominance in their study, with male-to-female ratio of 1:13 [19]. However, some authors recorded male predominance which was similar with the present study too (67%) [20-23]. The marked prevalence of the disease amongst young males in India may be attributed to an increased availability of commercially prepared areca nut preparations (pan masala) due to its easy access, effective price changes, and marketing strategies.

In this present study, semiquantitative evaluation was performed regarding histomorphometric parameters in NOM, OSFwd and OSFwtd. The current study recorded significant increase in the cell area in both OSFwd and OSFwtd in comparison to NOM [Table/Fig-5a,6]. The study recorded the mean of cellular major axis to be significantly increased in OSFwtd, while OSFwd was in-between NOM and OSFwtd [Table/Fig-5b,6]. The mean cellular minor axis demonstrated significant reduction in case of OSFwd when it is compared with NOM whereas it increased in OSFwtd [Table/Fig-5c,6]. aspect ratio showed gradual significant increase from NOM to OSFwtd and was highest in OSFwd [Table/Fig-5d,6].

aspect ratio is a parameter which denotes shape of an object and is inversely proportional to circularity. In other words, if aspect ratio increases the object will no longer be circular. The value of a perfect circle is equivalent to 1; and increase in the value indicates deviation from roundness and circularity.

The present study performed semiquantitative evaluation of the immunohistochemical features of the expression of E-cadherin in NOM; non dysplastic and dysplastic OSF in basal-suprabasal as well as spinous cell layers to assess the prognosis of the disease process and establish its role as a potential biomarker for assessing apico-basal polarity which is the key feature of EMT. The analysis was made in terms of gray scale values of E-cadherin expression which is inversely proportional to E-cadherin staining intensity.

The current study revealed the loss of membranous E-cadherin expression (inverse of gray scale value) in the tissue sections of NOM from basal to spinous cell layer. These observations are consistent with the finding of several other studies [27,29-32], which stated that E-cadherin expression is reduced in the spinous cells than basal, suprabasal layers in NOM suggesting the functional role of it in maintaining epithelial tissue integrity and loss of expression indicates a process of normal desquamation.

Das RK et al., reported that loss of membranous expression was highest in severe dysplasia [33]. Similar studies also found significant reduction in E-cadherin expression pattern sequence as disease progressed from NOM to oral epithelial dysplasia to malignancy [30,34]. The present study too recorded membranous E-cadherin expression to

be decreased significantly from NOM to non dysplastic to dysplastic OSF in both basal-suprabasal [Table/Fig-7a,8] and spinous cell layers [Table/Fig-7b,8].

While assessing the cytoplasmic E-cadherin expression, the present study revealed gradual decrease in this expression from NOM to diseased conditions both in basal-suprabasal [Table/Fig-7c,8] and spinous layer [Table/Fig-7d,8]. Although, significant difference of cytoplasmic expression between NOM and OSFwd was noted in both basal-parabasal and spinous layers however, the value was of considerable difference between NOM and OSFwtd.

The probable reason behind the above mentioned findings may be due to the fact that dyplastic features were not at all significant in OSFwtd. The study conducted by Anura A et al., (2014) revealed that the membranous nature of E-cadherin allows it to be expressed as dark brown lines that demarcate cell boundaries in oral epithelium. Both cytoplasmic and membranous expression was seen in the proliferative layer of NOM. However, there was a significant membranous expression than cytoplasmic with differentiation of cells towards supra layers. But in OSF, there was a notable loss of E-cadherin in the proliferative layer while, it was found to be more cytoplasmic in nature in the differentiative layers with reduced membranous expression. In OSFwtd and OSF with mild dysplasia, the expression of membranous E-cadherin in differentiative layers was comparable [35].

E-cadherin expression was evaluated by Nag R et al., in 2020 to ascertain OSF's oncogenic potential. They concluded that, there was breaking of E-cadherin junctions in OSFwd along with an increased cytoplasmic expression from NOM to OSFwtd to OSFwd. Decrease in membranous expression from normal to diseased condition was also noted [36].

Kalaimani G et al., (2023) observed in their study that E-cadherin expression was diminished with increasing grades of epithelial dysplasia, and OSF in comparison to that of NOM and finally concluded that E-cadherin expression is reduced as the disease progress towards malignancy [37].

Yamakanamardi B et al., (2023) while analysing immunohistochemical expression of E-cadherin in premalignant and malignant squamous lesions of the oral cavity, found out that as the grades of dysplasia increased and the disease progressed to malignancy, the expression of E-cadherin reduced [38].

The present study recorded membranous loss of E-cadherin molecule with concomitant cytoplasmic accumulation of the molecule, both in basal-parabasal and spinous layers of the surface epithelium. Therefore, loss of membranous E-cadherin and its subsequent accumulation in the cytoplasm of the cells can be regarded as a significant indicator in predicting the disease progression of OSF.

Limitation(s)

A small group of patients and controls were assessed which could be a probable reason behind all the results obtained by this study.

CONCLUSION(S)

The current study concluded that along with the histomorphometric parameters, immunohistochemical evaluation with molecular marker E-cadherin can be a value added diagnostic procedure in assessing the disease progression towards malignancy. However, further in depth studies with greater number of samples should be carried out in future.

Acknowledgement

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