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Original Article

Dentistry Section

Expressional Alteration of Cytokeratins in Oral Leukoplakia and Oral Leukoplakia-associated Oral Squamous Cell Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is characterised by high morbidity and poor survival rates, often developing from pre-existing Oral Potentially Malignant Disorders (OPMDs) like Oral Leukoplakia (OLKP). Early detection of a premalignant lesion can significantly increase survival rates. However, histopathological evaluation is unable to predict the potential for malignant transformation. This subjectivity has turned interest toward molecular markers, specifically Cytokeratins (CK), which may be essential in understanding the molecular changes occurring during the progression from OLKP to OSCC.

Aim: The aim of this study is to examine the Haematoxylin (H) and Eosin (E) stained light microscopic features of Normal Oral Mucosa (NOM), OLKP, and OSCC. We will compare and corroborate, the expressions of CK 8/18 and CK 5/6 in NOM, OLKP, and OSCC, and assess their roles as potential biomarkers in malignant transformation.

Materials and Methods: This cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology at Guru Nanak Institute of Dental Sciences and Research (GNIDSR), Kolkata, and the School of Medical Science and Technology (SMST), IIT, Kharagpur, West Bengal, India, from January 2019 to February 2020. The study included 26 cases of OLKP and OSCC, along with five cases of NOM. Histopathological and immunohistochemical evaluations (CK 8/18 and CK 5/6) were performed, measuring grey scale values using Image J software. Unpaired Student's t-test and one-way ANOVA tests were applied to better understand the significant changes in grey scale values, with a set significance level of <0.05.

Results: Out of total number participant, majority of them in both OLKP 9 (81%) and OSCC 12 (80%) patients were males. A considerable number of OLKP patients were in the age group of 51-60 years (35%), followed by the age range of 41-50 years (28%), 31-40 years (19%) and 61-70 years (18%), while in cases of OSCC-50% of patients were in the age group of 61-70 years, followed by the age range of 51-60 years (30%), 71-80 years (15%) and 41-50 years (5%). The staining intensity of both CK 5/6 and CK 8/18 significantly decreased (p-value <0.05) from basal to suprabasal and superficial layers in NOM and OLKP. Furthermore, a significant increase (p-value <0.05) in staining intensity was observed when comparing NOM vs. OLKP and NOM vs. OSCC.

Conclusion: It can be suggested that the expressional alterations of CK 8/18 and CK 5/6, as well as their correlation in different layers of NOM and OLKP, can be considered potential biomarkers for better understanding the progression toward OSCC, as well as its prognosis.

Keywords: Biomarkers, Immunohistochemistry, Malignant transformation, Oral cancer, Oral premalignant diseases

INTRODUCTION

Oral cancer accounts for 2%-4% of all cancer cases globally, with 90% of these classified as OSCCs [1]. The most significant risk factors for OSCC include tobacco and alcohol use [2]. Certain viral infections, such as Human Immunodeficiency Virus (HIV), Human Papillomavirus (HPV), Epstein-Barr Virus (EBV), and Hepatitis C Virus (HCV), are also correlated with a higher prevalence of OSCC [3]. Males are affected twice as often as females, primarily in the fifth decade of life [4]. The tongue and floor of the mouth are commonly affected, followed by the gingiva, palate, retromolar area alveolar ridge, buccal, and labial mucosa [2,5]. Clinically, OSCC can present as ulceroproliferative, exophytic, or endophytic growth of varying colors with indurated borders and lymphadenopathy [6,7]. Microscopically, features include islets and threads of malignant squamous epithelial cells invading the subepithelial connective tissue, with the formation of epithelial and/or keratin pearls, along with abnormal mitosis [4].

The incidence of OSCC has been observed in a significant number of individuals with a history of previously existing OPMDs like OLKP. The overall malignant transformation rate of this disease process ranges from 0.13% to 17% [8,9]. The chief etiological factor for Oral Leukoplakia (OLKP) includes the use of either smoked or smokeless tobacco. This condition is primarily found in individuals during the fifth to seventh decades of life [10] and occurs twice as commonly in males as in females [11,12]. Commonly affected sites include the buccal mucosa and commissural region, followed by the floor of the mouth, lateral border of the tongue, alveolar mucosa, and gingiva [13]. The clinical appearance of OLKP ranges from a whitish, fissured, to a wrinkled surface with sharply demarcated borders [8].

The characteristic histopathological features comprise epithelial hyperplasia, surface hyperkeratosis (hyperpara and/or hyperorthokeratosis), thickening of the spinous layer (acanthosis), loss of polarity of basal cells, broad and bulbous tear-drop shaped rete ridges, along with varying extents of epithelial dysplasia and loss of intercellular adherence [14].

Recently, various micro and macro molecular parameters have evolved to predict the malignant potential of OLKP. Among the several biological markers, Cytokeratins (CK) may be important for a better understanding of the molecular changes during this

transformation process. CK distribution within the epithelium is highly specific and varies with the extent of cellular differentiation [15]. Carcinomatous tissues express positivity for five CK types (CKs 7, 8, 18, 10, and 17) and CK19 in the basal and suprabasal layers of the epithelium [1].

An enhanced expression of CK 8 and 18 has been noted in tobacco-induced OLKP. Furthermore, low expression of CK 8 was observed on the healthy side in tumor border regions, whereas invasive squamous tumor cells showed a high expression level. Therefore, CK 8/18 serves as a marker for assessing altered cells in premalignant stages and early cancer [16]. Moreover, increased CK5 and CK6 staining expression was observed in about 81% of OSCC cases [16]. The expression of CK 5/6 was noted in well-differentiated cancers and not in normal squamous epithelium; however, it may be expressed during carcinogenesis [17].

Thus, studies of the expression of CK 8/18 and CK 5/6 in epithelial cells, along with knowledge of their association with cellular dysplasia, can be helpful in understanding the molecular scenario of this disease process and the progression of OLKPs towards OSCC. This understanding may ultimately improve diagnostic accuracy at an early stage, leading to better survival rates for oral cancer patients. Keeping this background knowledge in mind, this present semi-quantitative evaluation of the histopathological and immunohistochemical attributes of NOM, OLKP, and OSCC was conducted to assess their roles as potential biomarkers in the malignant transformation of OPMDs.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology at Guru Nanak Institute of Dental Sciences and Research (GNIDSR), Kolkata, and the School of Medical Science and Technology (SMST), IIT, Kharagpur, West Bengal, India, from January 2019 to February 2020. The entire study received ethical clearance from GNIDSR (GNIDSR/IEC/19-10 dated January 5, 2019).

Initially, seven healthy individuals and fifty patients with either OLKP or OSCC (OLKP associated) were selected and informed about the study to obtain their consent.

Inclusion criteria: Individuals aged 30-70 years without systemic medical disorders. Individuals with oral habits such as smoking, chewing tobacco, paan chewing, and chewing areca nuts with or without tobacco and lime were included.

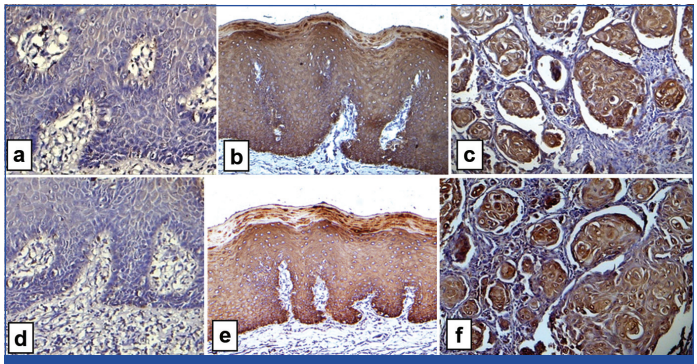
Exclusion criteria: Medically compromised patients were excluded from the study.

Sample size selection: After the primary selection, patients underwent routine medical and hematological investigations. Eleven patients had systemic complications and were therefore excluded. Nine patients and two healthy individuals declined to undergo biopsy; consequently, they were also excluded from the study. Additionally, four patients did not extend their consent for further diagnosis and treatment procedures. Finally, twenty-six patients (11 with OLKP and 15 with OSCC) and five normal individuals were selected for the study.

Study Procedure

The tissue samples were fixed in 10% neutral phosphate-buffered formalin for 24 hours with proper labeling. The fixed tissues were first dehydrated in ascending grades of alcohol and cleared in xylene, followed by embedding in molten paraffin (melting point 47-64°C).

Five-micron tissue sections were prepared using a Rotary Microtome (LEICA RM 2125 RT, Germany) and placed on both albumin-coated and poly-L-lysine coated slides. H and E staining, along with immunohistochemistry for CK 8/18 and CK 5/6, were performed. Evaluation of stained sections was conducted using a light microscope (Olympus CH20i with 10x and 40x objectives), with observations noted for NOM, OLKP, and OSCC [2]. Photomicrographs of these tissue sections from selected sites were captured using an upright microscope (LEICA DM750) fitted with a CCD camera (Moticam) [Table/Fig-1]. The epithelium, along with subepithelial connective tissue from sections of NOM and OLKP (areas with cellular and nuclear alterations and pleomorphism), was selected as Regions of Interest (ROI).



[Table/Fig-1]: Sample allocation in different groups.
Photomicrograph showing CK8/18 (a,b,c) and CK5/6 (d,e,f) in NOM (a,d), OLKP (b,e) and OSCC (c,f).

Then, thirty different ROIs were selected from NOM and OLKP, and corresponding fields were chosen from the IHC stained sections for further analysis based on the opinion of expert oncopathologists. In OSCC cases, due to the invasive nature of the tumor, the breach in the basement membrane, and the loss of the palisading pattern, cell layers could not be distinctly identified as basal, suprabasal, or superficial. In this scenario, cells from the tumor islands were considered for measuring IHC staining intensity.

From each of the thirty ROIs of the IHC stained sections, 50 cells were randomly selected from the basal layer, suprabasal layer, and superficial layers of NOM and OLKP sections. In the case of OSCC, 50 random cells were selected from the epithelial islands.

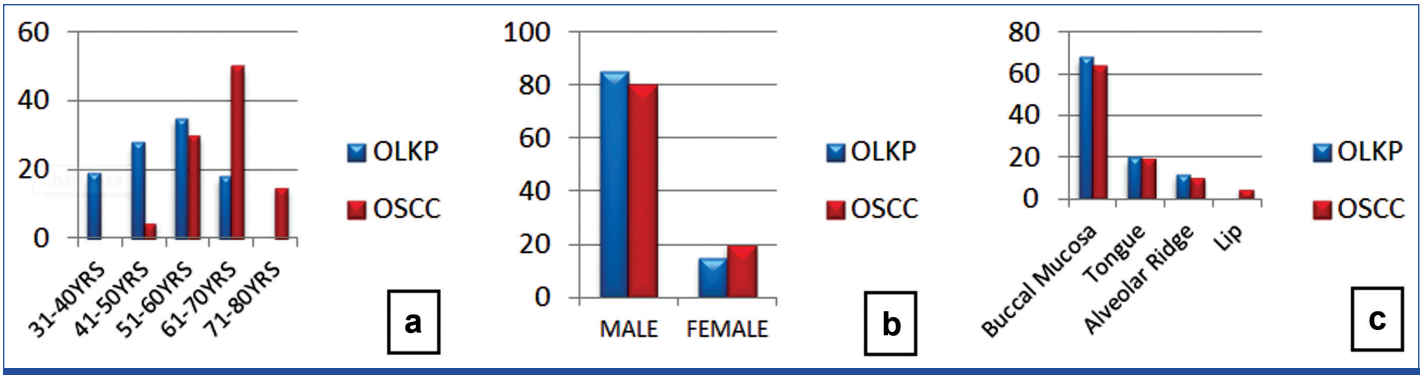
Using image analysis software (Image J {1.5a; Java 1.8.0_112 (64-bit); 4730K of 2991 MB (<1%)}, the molecular expression intensity of CK 8/18 and CK 5/6 was measured in different cell layers (basal, suprabasal, and superficial layers) in NOM and OLKP. The intensity of CK 8/18 and CK 5/6 stains was compared in the basal layer of NOM vs. OLKP, the suprabasal layer of NOM vs. OLKP, the superficial layer of NOM vs. OLKP, and OLKP vs. OSCC, in terms of mean gray scale value (the inverse of CK staining intensity).

STATISTICAL ANALYSIS

To assess statistical importance and significance, both descriptive and inferential statistics were performed. In descriptive statistics, the data was initially displayed using a bar diagram. Subsequently, scatter plots were drawn to visualise the placement of individual data in terms of minimum-maximum values and percentile distribution. For a better understanding of statistical significance, unpaired Student's t-tests and one-way ANOVA tests were performed.

RESULTS

Out of the total number participant, a considerable number of OLKP patients were in the age group of 51-60 years (35%), followed by 41-50 years (28%), 31-40 years (19%), and 61-70 years (18%). In cases of OSCC, 50% of patients were in the age group of 61-70 years, followed by 51-60 years (30%), 71-80 years (15%), and 41-50 years (5%) [Table/Fig-2a]. When considering the gender of the patients, the majority were male, with 9 (81%) in the OLKP group and 12 (80%) in the OSCC group [Table/Fig-2b]. Regarding the sites of involvement, most lesions in OLKP affected the buccal mucosa (68%), followed by the tongue (20%) and alveolar ridge (12%). In contrast, the majority of patients with OSCC had lesions present in the buccal mucosa and commissural area (64%), followed by the tongue (20%), alveolar ridge (11%), and lip (5%) [Table/Fig-2c].

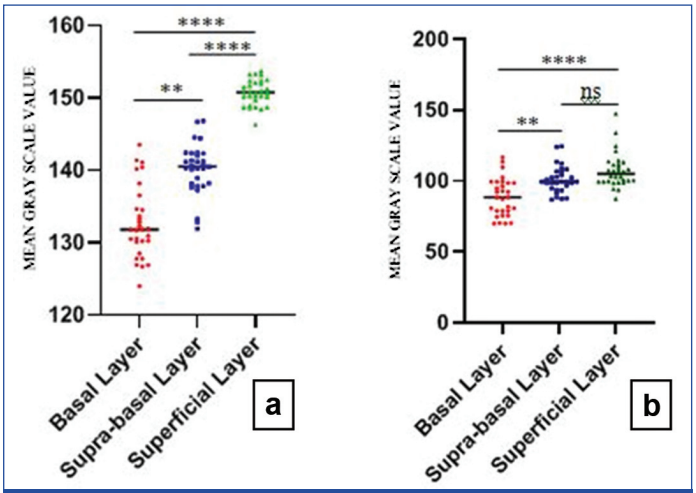


[Table/Fig-2]: Bar graph representation of distribution of OLKP and OSCC patients according to age groups (a), sex (b) and site (c).

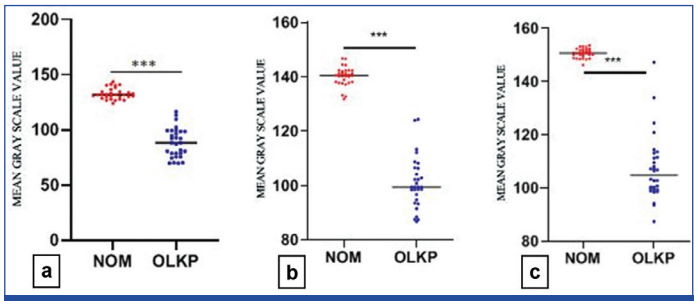
The majority of dysplastic features were absent in NOM, with only a few notable exceptions, including drop-shaped rete pegs (20%), nuclear pleomorphism (10%), enlarged nuclei (10%), and an increased nuclear/cytoplasmic (N/C) ratio (10%). In cases of OLKP, dysplastic features included drop-shaped rete pegs (80%), increased mitotic figures (70%), nuclear pleomorphism (70%), enlarged nuclei (80%), nuclear hyperchromatism (90%), atypical mitotic figures (60%), and an increased N/C ratio (80%). In OSCC epithelium, all dysplastic features were observed to be intense.

The molecular expression features of CK 8/18 and CK 5/6 in the basal cell layer, suprabasal cell layer, and superficial cell layer were extracted in terms of gray scale values in NOM, OLKP, and OSCC, and plotted in bar diagrams. Additionally, scatter plot graphs were presented for descriptive statistics.

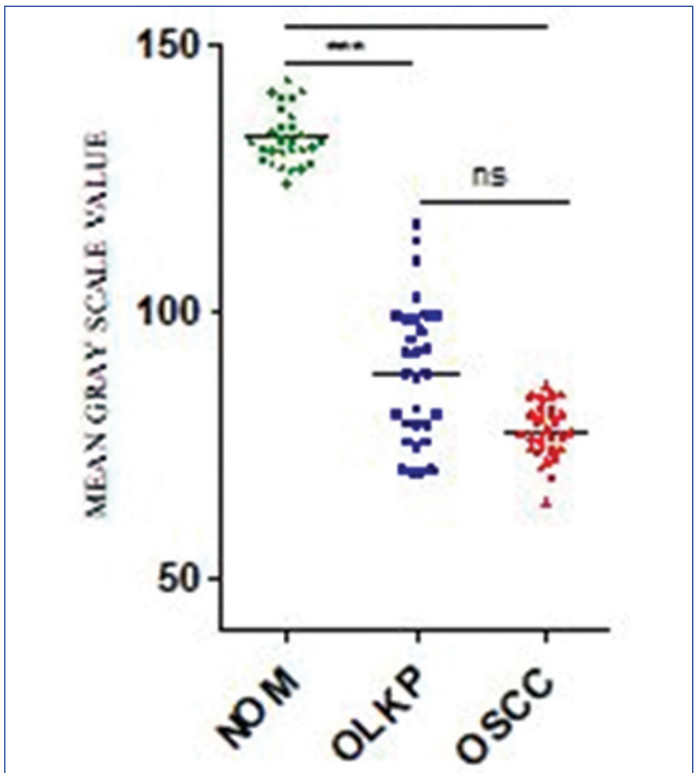
The intensity of CK 8/18 was statistically significant in the basal vs. suprabasal layer (p-value=0.0026), suprabasal vs. superficial layer (p-value <0.0001), and basal vs. superficial layer (p-value <0.0001) in NOM. In the case of OLKP, it was found to be statistically significant in the basal vs. suprabasal layer (p-value=0.0035) and basal vs. superficial layer (p-value <0.0001); however, there was no significant difference between the suprabasal vs. superficial layer (p-value=0.1417) [Table/Fig-3]. Additionally, when comparing the CK 8/18 staining intensity in NOM vs. OLKP, significant differences were found in the basal (p-value <0.0001), suprabasal (p-value <0.0001), and superficial layers (p-value <0.0001) of the epithelium [Table/Fig-4]. The mean grayscale values of NOM vs. OLKP (p-value <0.0001) and NOM vs. OSCC (p-value <0.0001) were also statistically significant. Furthermore, there was no statistical significance when comparing the mean values of CK 8/18 stained OLKP and OSCC (p-value=0.1033) [Table/Fig-5,6].



[Table/Fig-3]: The Scatter plot portrayed individual values of data with their mean values of CK 8/18 stain. a) Significant difference is noted in the staining intensity of CK 8/18 between the means of basal vs suprabasal layer, suprabasal vs superficial layer and basal vs superficial layer in NOM; & b) in OLKP, a significant difference was noted between the means of basal vs suprabasal layer and basal vs superficial layer but the difference is not significant in case of suprabasal layer when compared to superficial layer.



[Table/Fig-4]: The scatter plot represents a significant difference among the mean values of CK 8/18 staining intensity in basal layer (a), suprabasal layer (b) and superficial layer (c) of NOM and OLKP, respectively.

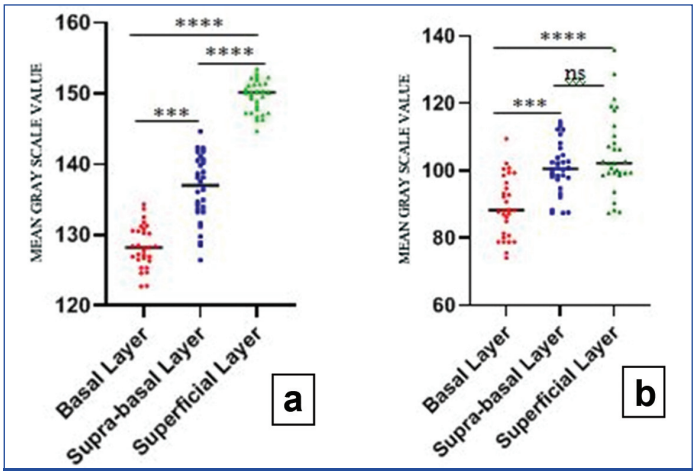


[Table/Fig-5]: The Scatter plot portrayed individual values of data with their mean values of CK 8/18 stain. Significant difference among the mean values of CK 8/18 staining intensity in NOM vs OLKP and NOM vs OSCC but the difference is statistically not significant in case of OLKP vs OSCC.

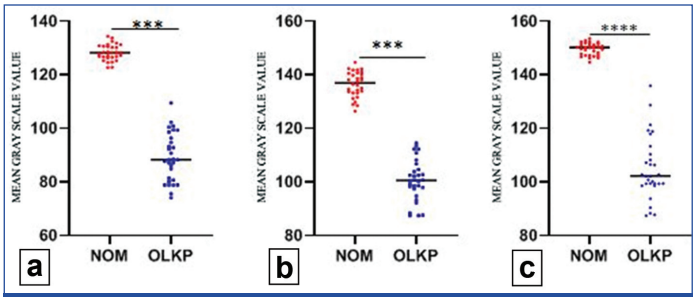
The intensity of CK 5/6 in OLKP samples was statistically significant in the basal vs. suprabasal layer (p-value <0.0001) and basal vs. superficial layer (p-value <0.0001); however, it was found to be non significant when comparing the suprabasal vs. superficial layer (p-value=0.8843) [Table/Fig-7]. Furthermore, when comparing the CK 5/6 staining intensity in NOM vs. OLKP in the basal (p-value <0.0001), suprabasal (p-value <0.0001), and superficial layers (p-value <0.0001) of the epithelium, as well as CK 5/6 intensity in NOM vs. OLKP (p-value <0.0001), NOM vs. OSCC (p-value <0.0001), and OLKP vs. OSCC (p-value <0.0001), all were statistically significant [Table/Fig-9,10].

Comparison between various conditions of surface epithelium at level cell layers stained with CK 8/18 along with their mean value±SD	Stasistically significant? (p-value <0.05, unpaired t-test)	Summary
Basal layer (132.7±0.9036) vs suprabasal layer (140.0±0.6403) in NOM	Yes (p-value: 0.0026)	**
Suprabasal layer (140.0±0.6403) vs superficial layer (150.7±0.3207) in NOM	Yes (p-value: <0.0001)	****
Basal layer (132.7±0.9036) vs superficial layer (150.7±0.3207) in NOM	Yes (p-value: <0.0001)	****
Basal layer (88.53±2.416) vs suprabasal layer(100±1.715) in OLKP	Yes (p-value: 0.0035)	**
Suprabasal layer(100±1.715) vs superficial layer(107.2±0.227) in OLKP	Not significant (p-value: 0.1417)	NS
Basal layer(88.53±2.416) vs superficial layer (107.2±0.227) in OLKP	Yes (p-value: <0.0001)	****
NOM (133±0.904) vs OLKP(88.5±2.42) in basal layer	Yes (p-value: <0.0001)	***
NOM(140±0.640) vs OLKP (101±1.72) in suprabasal layer	Yes (p-value: <0.0001)	***
NOM (151±0.321) vs OLKP(107±2.23) in superficial layer	Yes (p-value: <0.0001)	***
NOM (133±4.95) vs OLKP(88.5±13.2) mean value	Yes (p-value: <0.0001)	****
NOM(133±4.95) vs OSCC(77.6±5.16) mean value	Yes (p-value: <0.0001)	****
OLKP(88.5±13.2) vs OSCC(77.6±5.16) mean value	Not significant (p-value: 0.1033)	NS

[Table/Fig-6]: Comparison of p-values of CK 8/18 staining between NOM, OLKP and OSCC.
NS: not significant, *-**-***-****: significant



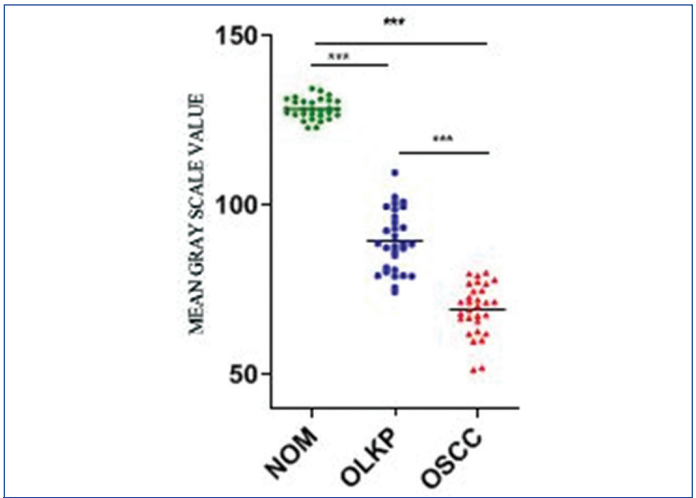
[Table/Fig-7]: The Scatter plot represents individual values of data with their mean values of CK 8/18 stain: (a) Significant difference was observed between the means of CK 8/18 stain in basal vs suprabasal layer, suprabasal vs superficial layer and basal vs superficial layer in NOM; &(b) in OLKP a significant difference among the mean values of basal and suprabasal layer and basal and superficial layer was noted whereas, the difference is not significant in case of suprabasal layer compared to superficial layer.



[Table/Fig-8]: The Scatter plot depicted that there is a significant difference between the mean value of CK 5/6 staining intensity in basal layer (a), suprabasal layer (b) and superficial layer (c) of NOM and OLKP.

DISCUSSION

In the present research study, a clinicoepidemiologocal evaluation of OLKP and leukoplakia-associated OSCC was conducted based on parameters such as age, sex, site, and clinicopathological evaluation through light microscopy. The H&E stained light microscopic features of NOM, OLKP, and OSCC were integrated with IHC (CK 8/18 and CK 5/6) features to compare and corroborate the progression towards malignancy. Liu W et al., stated that the peak incidence of OLKP is mainly recorded in the fifth decade of life [18]. However, in India, a peak incidence was noted between 35-45 years of age (Rajendran R) [19]. Regarding OSCC, Kordek R et al., stated that this disease is most commonly diagnosed in the sixth decade of life [20]. Later, Warnakulasuriya S et al., observed that the chance of oral cancer increases with age, and the majority of cases occur in



[Table/Fig-9]: The Scatter plot portrayed individual values of data with their mean values of CK 8/18 staining intensity. A significant difference among the mean values of CK 8/18 stain in NOM, OLKP and OSCC.

Comparison between various conditions of surface epithelium at level cell layers stained with CK 5/6 along with their mean value±SD	Statistically significant? (p-value <0.05, unpaired t-test)	Summary
Basal layer (128.3±0.5461) vs suprabasal layer (136.4±0.8664) in NOM	Yes (p-value=0.0006)	***
Suprabasal layer (136.4±0.8664) vs superficial layer (149.6±0.4164) in NOM	Yes (p-value <0.0001)	****
Basal layer (128.3±0.5461) vs superficial layer(149.6±0.4164) in NOM	Yes (p-value:<0.0001)	****
Basal layer (89.23±1.645) vs suprabasal layer (100.7±1.408) in OLKP	Yes (p-value=0.0001)	***
Suprabasal layer(100.7±1.408) vs superficial layer(104.9±2.140) in OLKP	Not significant (p-value: 0.8843)	NS
Basal layer (89.23±1.645) vs superficial layer (104.9±2.140) in OLKP	Yes (p-value: <0.0001)	****
NOM (128±2.99) vs OLKP (89.23±9.01) in basal layer	Yes (p-value: <0.0001)	****
NOM(136.4±o.8664) vs OLKP (100.7±1.408) in suprabasal layer	Yes (p-value: <0.0001)	****
NOM (149.6±0.4164) vs OLKP (104.9±2.140) in superficial layer	Yes (p-value: <0.0001)	****
NOM (128±2.99) vs OLKP (89.2±9.01) mean value	Yes (p-value: <0.0001)	****
NOM (128±2.99) vs OSCC (69.1±7.57) mean value	Yes (p-value: <0.0001)	****
OLKP (89.2±9.01) vs OSCC (69.1±7.57) mean value	Yes (p-value: <0.0001)	****

[Table/Fig-10]: Comparison of p-values of CK 5/6 staining between NOM, OLKP and OSCC.
NS: not significant, *-**-***-****: significant

people aged 50 years or older [21]. In the present study, too, most OLKP patients (35%) belonged to the age group of 51-60 years, while OSCC patients (50%) were in the 61-70 years age range.

Thus, the present study strongly supports the observations made by earlier researchers. Various studies have noted a strong male predilection for OLKP (Mehta FS et al., Bánóczy, Gupta PC et al.,) [22-24] and OSCC (Warnakulasuriya S et al., Sawlani K et al.,) [25,26] worldwide. Similar observations were noted in the present study, which found a strong male predilection for OLKP (85%) and OSCC (80%). Hence, it can be concluded that both OLKP and OSCC are more common among males.

According to previous studies (Axéll T et al., Brouns ER et al.,), OLKP commonly presents on the buccal mucosa, alveolar ridge, tongue, floor of the mouth, lower lip, and hard palate [27,28]. The most frequently involved sites are reported to be the buccal mucosa and commissures, followed by the floor of the mouth, tongue, alveolar mucosa, and gingiva [29]. The present study also revealed that the most commonly affected sites in OLKP (68%) and OSCC (64%) were the buccal mucosa and commissural area. This is consistent with the observations made by previous researchers. Furthermore, in the present study, while evaluating light microscopic H&E stained tissue sections, dysplasia was found to be the most consistent feature in OLKP. Features such as drop-shaped rete pegs (80%), increased mitotic figures (70%), nuclear pleomorphism (70%), enlarged nuclei (80%), nuclear hyperchromatism (90%), atypical mitotic figures (60%), and an increased nuclear/cytoplasmic (N/C) ratio (80%) were primarily observed. However, most dysplastic features were absent in NOM, with only a few features like drop-shaped rete pegs (20%), nuclear pleomorphism (10%), enlarged nuclei (10%), and an increased N/C ratio (10%) present. When examining the invasive epithelial pearls in cases of OSCC, dysplastic features were noted to be pronounced. Therefore, it can be concluded that dysplastic features are most prominent in OSCC, followed by OLKP, while NOM is relatively free from any notable dysplastic features.

A semi-quantitative evaluation of IHC (CK 8/18 and CK 5/6) features was performed to analyse the expressional alterations in NOM, OLKP, and OSCC in the basal, suprabasal, and superficial layers in terms of grayscale value. In this study, a significant gradual decrease in the staining intensity of CK 8/18 was observed from the basal to suprabasal layers (p-value: 0.0026), from the suprabasal to superficial layers (p-value <0.0001), and from the basal to superficial layers (p-value <0.0001) in NOM. In OLKP, a statistically significant decrease was observed from the basal to the suprabasal layers (p-value=0.0035), while a slight decrease was noted from the suprabasal to superficial layers (p-value: 0.1417). Additionally, a statistically significant increase in CK 8/18 staining intensity was observed between the basal layers (p-value <0.0001), suprabasal layers (p-value <0.0001), and superficial layers (p-value <0.0001) when comparing NOM to OLKP. Moreover, a significant increase in CK 8/18 staining intensity from NOM to OSCC (p-value <0.0001) was noted in the present study. However, when comparing CK 8/18 intensity in OLKP to OSCC, there was no significant difference (p-value=0.1033).

According to Nanda KD et al., no CK expression was noted in NOM with CK 8/18, whereas basal and suprabasal staining was observed in OLKP. The intensity of staining expression was mild in the basal layer in 40% of leukoplakia cases, while mild staining was noted in 10% of leukoplakia cases in the suprabasal layer [30]. Kale D et al., also reported that no immunoreactivity of CK 8/18 was noted in the oral mucosa of non-neoplastic cases [31]. Sharda S Sawant et al., reported a highly positive (98%) expression of CK 8/18 in OSCC, with most carcinomatous tissues exhibiting de novo expression of five CKs (CKs 7, 8, 18, 10, and 17) and CK 19, both in the basal and suprabasal layers [32]. Another study conducted by Jaiswal P et al., observed a significant increase in the staining expression of CK 8/18 in OSCC and OLKP with dysplasia [33]. The observations made by various researchers align with the results of the present study. Thus, it can be proposed that there is a significant increase in the staining intensity of CK 8/18 in OLKP compared to NOM in the basal and suprabasal cell layers.

Crook T et al., highlighted a strong expression of CK5 and CK6 in squamous cell carcinoma in their study [34]. Kaufmann O et al., demonstrated high staining expression of CK5 and CK6 in 81% of squamous cell carcinomas [35]. Alam H et al., stated that CK 5/6, CK-10, and CK-14 are expressed in well-differentiated cancers with lower hypoxia and cell cycle deregulation and are not physiologically expressed in normal squamous epithelium, but may be expressed during carcinogenesis [17]. In the present study, a significant gradual decrease in the staining intensity of CK 5/6 was noted from the basal to suprabasal layers (p-value=0.0006) and also from the suprabasal to superficial layers (p-value <0.0001) in NOM. In the case of OLKP, a significant difference was observed between basal to suprabasal layers (p-value=0.0001) and basal to superficial layers (p-value <0.0001), but this difference was not significant when comparing the suprabasal and superficial layers (p-value=0.8843). However, a statistically significant increase in CK 5/6 staining intensity from NOM to OLKP was noted in the basal layers (p-value <0.0001), suprabasal layers (p-value <0.0001), and superficial layers (p-value <0.0001) as well.

Moreover, in the present study, there was a significant gradual increase in CK 5/6 expression from NOM to OLKP and OSCC. Thus, various reports from previous authors are in accordance with the results of the present study. Therefore, it can be opined that there is a significant increase in the staining intensity of CK 5/6 in OLKP compared to NOM in the basal and suprabasal cell layers.

Limitation(s)

The sample size was small; hence, studies with larger sample sizes in the future are recommended.

CONCLUSION(S)

CK 8/18 and CK 5/6 can be considered potential biomarkers to assess the malignant transformation of OLKP. Further intensive studies with an increased number of study subjects and observations of the expressional alterations within different cell layers of the epithelium in different grades of dysplasia should be performed in a more integrated manner for a better understanding of this disease process and its potentiality to express progression toward malignancy.

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