

## Original Article

# Oral leukoplakia: Transmission electron microscopic correlation with clinical types and light microscopy

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## ABSTRACT

**Background:** Leukoplakia, is a precancerous lesion that is most commonly encountered in the oral cavity. The grade of dysplasia is presumed to be the most important indicator of malignant potential. There are many promising aspects in advanced methods for the evaluation of oral precancer and cancer. Among these methods, electron microscopic examination predicts the true biologic potential more accurately than conventional histology and has some success in the early detection of potentially malignant lesions. It has been reported in the literature that there is some correlation between clinical, histopathological, and transmission electron microscopic features.

**Materials and Methods:** In this cohort study (prospective research), from the total of 9 subjects, 3 had homogenous leukoplakia, 3 had ulcerative type of oral leukoplakia, and 3 had nodular type of oral leukoplakia. Two patients were selected as control patients. Transmission electron microscopic examination was carried for all the cases and controls. All the findings were correlated with clinical features and light microscopy.

**Results:** Clinically and histologically, mild leukoplakia showed break in basement membrane, which can only be observed under transmission electron microscope (TEM). Additional dysplastic features were observed under transmission electron microscope, which are indicative of neoplastic process.

**Conclusions:** Thus, it is finally concluded that nodular leukoplakia seems to be the most severe clinical type of leukoplakia showing highest risk of malignant transformation. Homogenous leukoplakia might show break in basement membrane under TEM.

**Key Words:** Epithelial dysplasia, oral leukoplakia, pathologic cytoplasmic process, transmission electron microscope

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## INTRODUCTION

Leukoplakia is a precancerous lesion, which is most commonly encountered in the oral cavity. The term literally means a white patch, which was first described by Hungarian dermatologist, Erno Schwimmer in 1877. Despite major advances in the

molecular pathology of head and neck cancer (HNC) and oral cancer, there remain numerous gaps in our knowledge of the molecular markers involved in oral carcinogenesis.

Oral squamous cell carcinomas (OSCCs) appear to have a multifocal character, with half of them developing on the same site as a previous leukoplakia. Complex molecular mechanisms are implicated and the identification of a single marker to predict outcomes in all oral premalignant lesions remains a difficult challenge.

Oral precancerous lesions are usually histologically classified by the presence or absence of oral epithelial dysplasia, Nevertheless, no objective methods are

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yet available to typify dysplastic lesions and allow consistent and reproducible results to be obtained, a number of studies in recent years have been conducted to develop cellular and molecular markers capable of indicating the risk of malignant transformation of dysplastic epithelium and to predict behavior over time, something which cannot be done effectively with degree of dysplasia.<sup>[1]</sup>

During the evaluation of clinical features of leukoplakia, three types were recognized. This classification has been proposed by Mehta *et al.* in 1993 and also accepted by Prabhu *et al.* 1996 in his book, "Oral diseases in tropics".<sup>[2,3]</sup>

1. Homogenous leukoplakia (Simplex type)
2. Ulcerative leukoplakia (Erosive type)
3. Nodular leukoplakia (Speckled type)

Among the three clinical types nodular leukoplakia shows a higher frequency (Mehta F.S.)<sup>[2]</sup> Characteristic histological alteration had already been determined in previous studies for each clinical type.<sup>[4,5]</sup> But documentation of ultrastructural details of clinical types of leukoplakia is quite rare. In this study, attempt was made to correlate histopathological and ultrastructural findings in the three clinical types of leukoplakia.

## MATERIALS AND METHODS

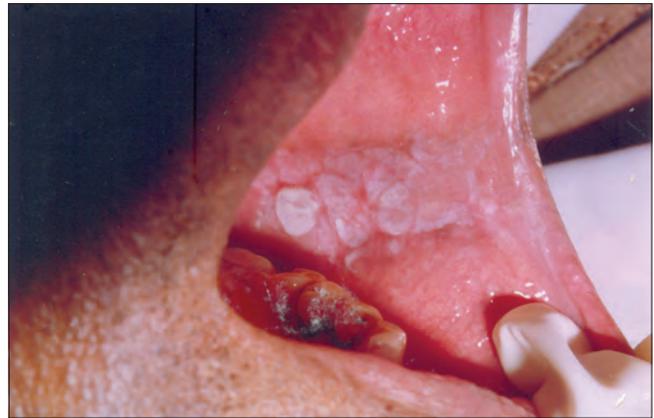
From the total of 9 subjects included in our study, 3 had homogenous leukoplakia, 3 had ulcerative type of oral leukoplakia, and 3 had nodular type of oral leukoplakia. Two patients were selected as control patients [Figures 1-3].

The biopsy specimens were cut longitudinally into two halves. Larger part was kept for routine histopathological studies and the other part for TEM study, conducted as per the procedure given by Bancroft.<sup>[6]</sup>

### Transmission electron microscope examination/ reporting

The grids were loaded and viewed under JEOL 1200 EX II transmission electron microscope. Electronmicrographs were taken of selected areas.

The transmission electron microscopic findings were recorded, evaluated, corroborated, and analyzed keeping in mind the aforesaid findings in reference to aims and objective of the conducted study. In view of small sample size of each type of leukoplakia, statistical analysis was not used in this study.



**Figure 1:** Clinical photograph showing homogenous leukoplakia of buccal mucosa



**Figure 2:** Clinical photograph showing ulcerative leukoplakia of commissure



**Figure 3:** Clinical photograph showing nodular leukoplakia of commissure

## OBSERVATIONS AND RESULTS

Clinical features of 3 types of oral leukoplakia, their light microscopic, and TEM findings were eventually evaluated [Figures 1-3].

**Transmission electron microscopic features**

These following ultrastructural features were considered to be characteristic of dysplastic changes in the epithelium where malignant transformation is likely to occur. These features are:

1. Discontinuous basal lamina
2. Ruptured hemidesmosomes
3. Presence of pathologic cytoplasmic processes

Altered keratinization -

4. Decreased tonofilaments
5. Decreased keratohyaline granules
6. Decreased odland bodies
7. Widened and disrupted intercellular junctions.
8. Presence of intracellular vacuolization.
9. Nuclear alterations.
10. Nucleolar alterations
11. Degenerated mitochondrias in suprabasal layers.
12. Increased ribosomes and their aggregation in the form of “rosette”.

All the above findings were noted in the respective clinical types.<sup>[5]</sup>

**Correlation of clinical types of leukoplakia with light and electron microscopic findings**

The important ultrastructural features, which were observed in the overlying epithelium, were compared with light microscopic features in each clinical type of 9 cases of oral leukoplakia [Tables 1-3].

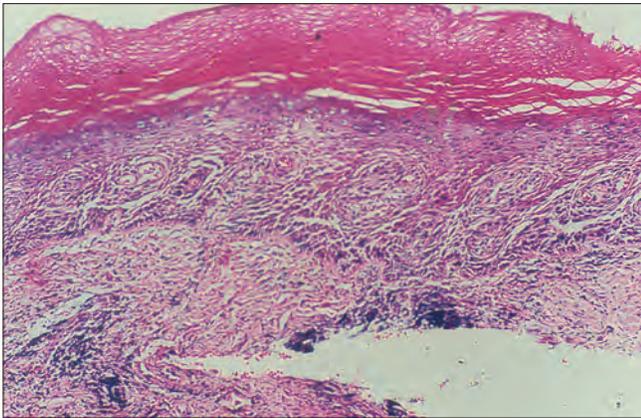
Under light microscope all the cases of ulcerative and nodular type of leukoplakia showed discontinuity of basement membrane at places [Figures 4 and 5]. This finding was confirmed by TEM, showing multiple and clearly discontinuous basal lamina, ruptured hemidesmosomes, along with the presence of pathologic cytoplasmic processes [Figure 6], whereas in cases of homogenous leukoplakia none of them showed broken basement membrane [Figure 7] under light microscope but when viewed under TEM basal lamina was not intact in two cases [Figures 8 and 9]. So these two cases of homogenous leukoplakia, which seemed to be innocuous under light microscope were found to be not so, when viewed under TEM.

Under light microscope, only one case homogenous leukoplakia showed altered keratinization in the form of intraepithelial keratin pearl formation [Figure 10], while under TEM, all the cases of homogenous leukoplakia showed dyskeratosis in the form of thick tonofilament bundles arranged around the nucleus and covered by ribosomes along with increased and irregularly shaped keratohyaline granules [Figure 11].

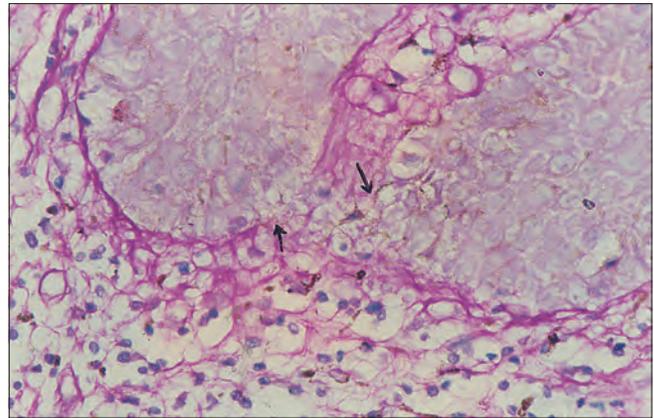
**Table 1: I-Transmission electron microscopic features of leukoplakia**

Biopsy no.	Basal lamina	Hemidesmosomes	Pathologic cytoplasmic processes	Nuclear changes	Nucleolus	Mitochondria	Ribosomes (even distribution)	Intercellular junctions	Intracellular vacuolization	Tonofilaments	Keratohyaline granules of varying size	Odland bodies
Homogenous leukoplakia												
5856/98	Intact	Intact	-	Indented	Multiple	Degeneration	Even	Broken	-	Bundles	Varying size	Odland bodies
5893/98	Broken	Ruptured	PCP	Indented	Multiple	Degeneration	Even	Wide and broken	Vacuolization	Bundles	Varying size	Odland bodies
5900/98	Broken and Multiple	Ruptured	PCP	Indented	Multiple	Degeneration	Even	Broken	-	Bundles	Varying size	Odland bodies
Ulcerative leukoplakia												
5861/98	Broken and multiple	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	Vacuolization	Dispersed	Varying size	Odland bodies
5736/98	Broken and multiple	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	-	Dispersed	-	-
5837/98	Broken and multiple	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	Vacuolization	Dispersed	-	-
Nodular leukoplakia												
5867/98	Broken and multiple	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	-	Dispersed	-	-
5910/98	Broken and multiple	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	Vacuole	Dispersed	-	Odland bodies
5851/98	Broken	Ruptured	PCP	Indented and SD	Multiple	Degeneration	Even	Broken	vacuole	Dispersed	-	Odland bodies

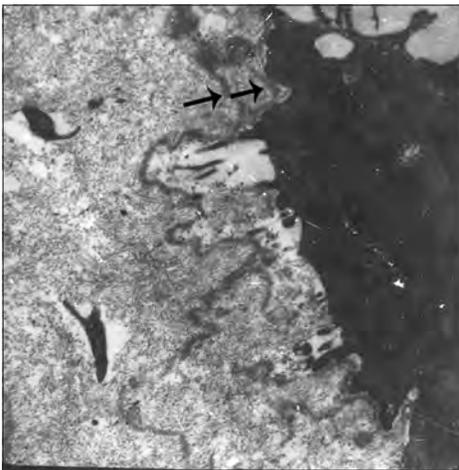
HP: Hyperplastic; BH: Basilar hyperplasia; HC: Hyperkeratinization; Multi: Multiple pathologic cytoplasmic processes (PCP); Mod: Moderate; SD: Nuclear membrane sinusoidal dilatation



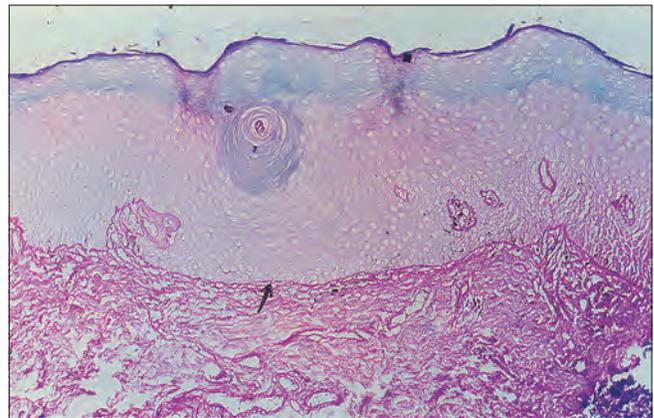
**Figure 4:** Photomicrograph of ulcerative leukoplakia shows moderate dysplasia (H and E, x10)



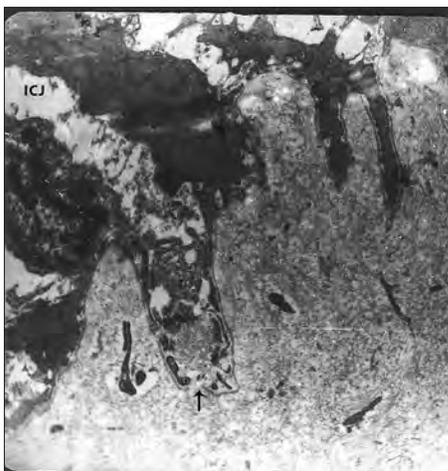
**Figure 5:** Photomicrograph of ulcerative leukoplakia shows interrupted basement membrane (PAS x40)



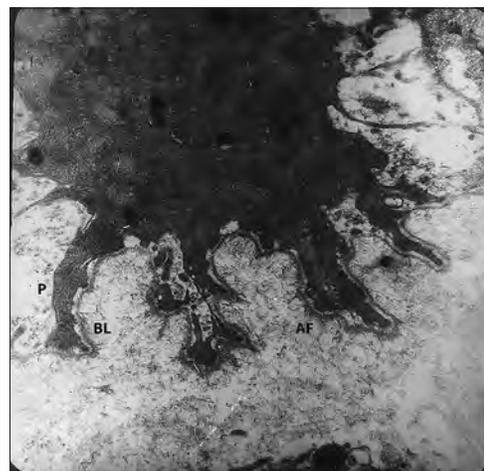
**Figure 6:** Electron micrograph of nodular leukoplakia shows multiplication of basal lamina suggestive of precancerous changes (x3000)



**Figure 7:** Photomicrograph shows homogenous leukoplakia showing intact basement membrane (PAS x10)



**Figure 8:** Electron micrograph of homogenous leukoplakia shows discontinuous basal lamina and broken intercellular junctions (ICS) (x2500)



**Figure 9:** Electron micrograph of homogenous leukoplakia shows pathologic cytoplasmic processes suggestive of preneoplastic process (P) (x3250)

On the contrary, all cases of ulcerative and nodular leukoplakia showed decreased keratinization by

showing decreased and dispersed tonofilaments, with very few or absence of keratohyaline granules. Thus, all the above features epithelial dysplasia can only be seen under TEM.



**Table 3: History and clinical features**

File no.	Biopsy no.	Age/sex	Religion	Socio-economic status	Site of lesion	Dietary habits	Tobacco+lime quid keeping	Smoking tobacco	Drinking	Teeth cleaning	Asymptomatic unawares	Discomfort	pain	Burning sensation
Homogenous leukoplakia														
	89	5856/98	39/M	Hindu	Middle class	RBM	Veg/spicy	4-5 times/day since 15 yrs	-	Tooth paste	-	+	-	-
	91	5893/98	58/M	Christian	Middle class	LBM	Mixed/Non-spicy	2-3 times/day since 2-3 yrs	Cigarette 4-5 packets/day	Colgate paste	+	-	-	-
	96	5900/98	27/M	Hindu	Middle class	LBM	Mixed/spicy	4-5 times/day since 8-9 yrs	Once/week	Colgate paste	+	-	-	-
Ulcerative Leukoplakia														
	90	5861/98	58/M	Hindu	Middle class	RBM+Xommissure	Mixed/spicy	-	12-15 bidis/day since 25 yrs	Occasionally Colgate paste	-	+	+	+
	85	5736/97	55/F	Hindu	Lower class	Gingival-lower left anterior	Veg/spicy	-	4-5 times/day since 15 yrs	-	-	+	+	+
	86	5837/98	41/M	Hindu	Middle class	RBM	Veg/spicy	5-6 times/day since 17-18 yrs	2-3 times per week	Tooth paste	-	+	+	+
Nodular leukoplakia														
	90	5867/98	58/M	Hindu	Middle class	LBM+Xommissure	Mixed/spicy	12-15 bidies/day since 25 yrs 1 pack/day	Occasionally	Colgate	-	+	+	+
	96	5910/98	54/M	Hindu	Lower middle class	RBM+ABM	Mixed/spicy	4-5/day	Once/day	Snuff since 15-20 yrs	-	+	+	+
	99	5851/98	75/F	Christian	Middle class	RBM+Lower gingival	Mixed/non-spicy	5-6 times/day since 15-20 yrs	-	-	-	+	+	+

RBM: Right buccal mucosa; LBM: Left buccal mucosa

While all the cases of homogenous leukoplakia showed intact intercellular junctions under light microscope, but showed widened and disrupted intercellular junction under TEM, thus again stressing the importance of TEM over light microscope.

Two cases each, of ulcerative and nodular leukoplakia, while only one case of homogenous leukoplakia revealed intracellular vacuolization under light microscope. This finding was confirmed under electron microscope as 'Laking effect' [Figure 14].

All the 9 cases of leukoplakia showed varying degree of nuclear alterations under light microscope. Homogenous leukoplakia showed very few abnormal mitotic figures only in basal cell layer. This finding was confirmed under TEM as mild nuclear indentation in basal and suprabasal layer.

On the contrary, nodular and ulcerative leukoplakia showed many abnormal and few bizarre mitotic figures in both basal and suprabasal layers, which is also confirmed by TEM showing moderate to severe nuclear indentations. In addition, one case of ulcerative and one case of nodular type shown the nuclear indentation also in spinous cell layer [Figure 15], an important finding which was not observed under light microscope indicating high malignant potential.

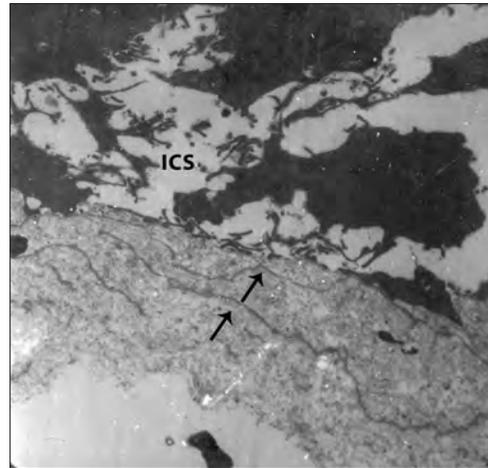
All the cases of ulcerative and nodular leukoplakia showed nucleolar margination in which the nucleoli were situated towards the nuclear membrane, which is an important feature of neoplastic change seen in growth phase of benign tumors.

Thus, this nuclear alteration, an important preneoplastic feature can only be observed under TEM.

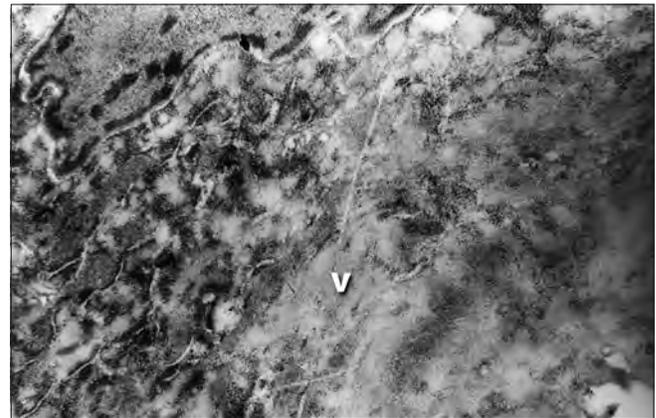
The degenerated mitochondria and increased ribosomes and their aggregation in the form of rosette, are the features which cannot be observed under light microscope, but can be observed under TEM.

Normally mitochondria are seen with distinct cristae in all the layers of epithelium. In three cases of homogenous leukoplakia, degeneration of mitochondria in the form of cristolysis and fused and disoriented cristae was seen only in suprabasal layer. Whereas in ulcerative and nodular type, such change in mitochondria was seen in all the layers of epithelium [Figure 16].

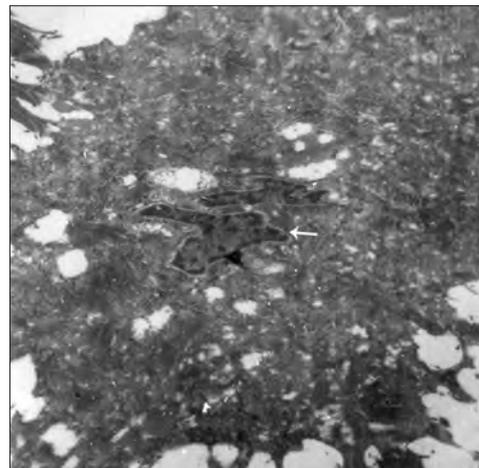
All the cases of leukoplakia showed increased number of ribosomes with even distribution, indicating some degree of dysplasia. However, the rosette formation,



**Figure 13:** Electron micrograph of nodular leukoplakia shows multiplication of basal lamina (→) and severely broken intercellular junctions (ICS) (x2500)



**Figure 14:** Electron microscopy of leukoplakia shows intracellular vacuolization in the form of "laking effect" (V) suggestive of preneoplastic change (x10,000)



**Figure 15:** Photomicrograph of nodular leukoplakia shows severe nuclear indentations suggestive of precancerous change (x2500)

indicative of severe dysplasia was not seen in any of the cases. Another important nuclear change which cannot be observed under light microscope is the sinusoidal dilatation between the two lamellae of nuclear membrane [Figure 17]. This alteration was observed in all cases of ulcerative and nodular leukoplakia.

All the cases of leukoplakia showed multiple nucleoli under light microscope except one case of homogenous leukoplakia. But under TEM all cases of leukoplakia showed multiple nucleoli of varying size and shape.

## DISCUSSION

Histopathologically, oral epithelial dysplasia currently is the most important prognostic indicator for determining the malignant transformation risk of oral leukoplakia.<sup>[7,8]</sup>

Various studies have been carried out to correlate histopathological features of oral leukoplakia with clinical features but no conclusions have been obtained as similar clinical types may show different histopathological grade of dysplasia.<sup>[8,9]</sup>

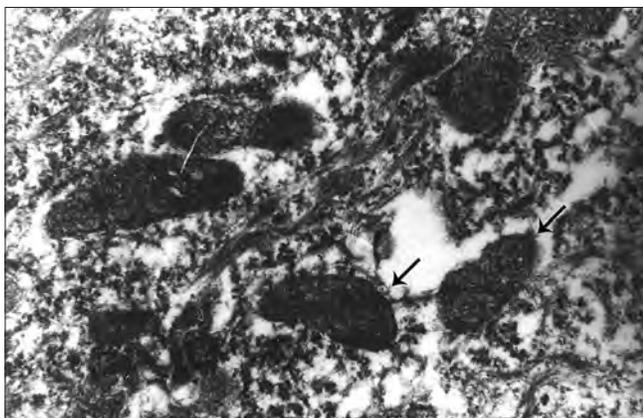
Transmission electron microscopy (EM) has been used to identify the ultrastructural details of normal, precancer and cancerous human oral mucosa. However, inconsistent reports of structural descriptions have rendered transmission EM valueless in the diagnosis as a prognostic indicator, therefore various authors have studied normal mucosa, severe dysplasia, oral SCC, and normal margin adjacent to oral SCC for features of dysplasia to compare the ultrastructural features of normal and premalignant oral mucosa and oral SCC. for diagnostic purposes.<sup>[9-15]</sup>

Therefore, this study was designed to study epithelial dysplasia under TEM and to establish a possible correlation between clinical types and light microscopy with TEM features, as relative paucity of such correlative studies in the literature.

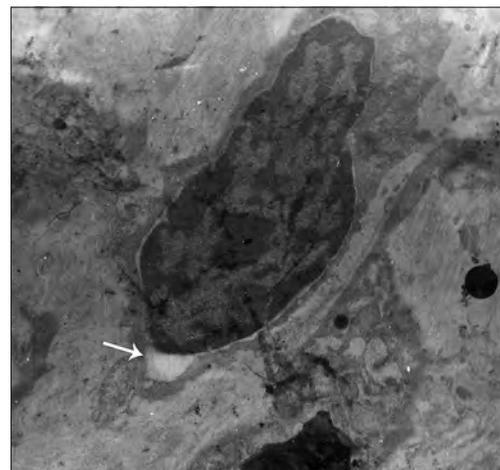
Under light microscopy ulcerative and nodular leukoplakia showed interrupted basement membrane, which was confirmed under electron microscope by discontinuous basal lamina at places, ruptured hemidesmosomes, and presence of pathologic cytoplasmic processes.<sup>[16]</sup> On the contrary, all cases of homogenous leukoplakia showed intact basement membrane but ultrastructurally all the above features were seen only in 2 cases. Thus, showing the importance of transmission electron microscopic study.<sup>[10,14,17-23]</sup>

In the landmark study of Banoczy *et al.* (1980),<sup>[16]</sup> ulcerative and nodular leukoplakia showed decreased keratinization in the form of decrease and dispersed tonofilaments, few keratohyaline granule and odland bodies, characteristics of parakeratosis. Similar findings were observed in the present study in ulcerative and nodular type whereas in one case of homogenous leukoplakia, intraepithelial pearl formation was seen under light microscope indicating increased keratinization, which is confirmed electron microscopically as thick bundles of tonofilaments, arranged circumferentially around the nucleus, irregularly shaped numerous keratohyaline granules and odland bodies.<sup>[12,13,17,24-26]</sup>

Under light microscope only ulcerative and nodular types showed disrupted intercellular junctions, which was confirmed under TEM as ruptured desmosomes



**Figure 16:** Electron micrograph of ulcerative leukoplakia shows diffuse and disoriented cristae of mitochondria suggestive of neoplastic change (×10,000)



**Figure 17:** Electron micrograph of ulcerative leukoplakia shows sinusoidal sac between two lamellae of nuclear membrane (×10,000)

and widened intercellular spaces. This finding accords well with that of Banoczy *et al.* (1980).<sup>[4]</sup> Along with this, all cases of homogenous leukoplakia also showed disrupted intercellular junctions, which was not seen under light microscope, suggesting importance of TEM.

Silverman *et al.*<sup>[19]</sup> suggested that intracellular vacuolization, which is a degenerative process, observed under light microscope as one of the dysplastic features appear as a 'laking effect' electron microscopically. Most of the cases of ulcerative and nodular leukoplakia showed this finding under both light and TEM.

Nuclear alterations in the form of nuclear hyperchromatism, nuclear pleomorphism, and abnormal mitoses were seen under light microscope. These findings were confirmed by TEM, which showed mild nuclear changes in homogenous and moderate to severe nuclear alterations in ulcerative and nodular leukoplakia in the form of indented nuclei and sinusoidal dilatation of nuclear membrane. Similar findings were seen by Banoczy (1980).<sup>[4,17,21,26-28]</sup>

According to Ghadially *et al.* (1980), in malignancy the nucleoli shows various alterations like varying size and shape, multiplicity, and nucleolar margination. All these features are seen in growth phase of tumors. Light microscope shows only the presence of multiple nucleoli, whereas transmission electron microscopy reveals the further structural details in the form of increased nucleoli of varying size and shape with nucleolar margination. These features are suggestive of malignant change found in ulcerative and nodular leukoplakia in our study.<sup>[11]</sup>

Degenerated mitochondria and increased ribosomes are the important features, which are indicative of dysplastic changes<sup>[18]</sup> and can only be seen under TEM.<sup>[29]</sup>

Ultrastructural features of epithelial dysplasia in oral leukoplakia closely resemble oral carcinoma, which cannot be seen under light microscopy proves the importance of TEM.<sup>[24,29,30-34]</sup>

Many epithelial dysplastic features, which are seen under light microscope can also be seen under TEM but with a clear view of every structural detail. In addition to this, there are some other important dysplastic features of the epithelium, which can only be observed under TEM and are suggestive of neoplastic transformation and varied prognosis of same type of lesion.

These ultrastructural are features indicative of epithelial dysplasia are discontinuous of basal lamina, ruptured hemidesmosomes, presence of pathologic cytoplasmic processes, decreased tonofilament, keratohyaline granules and odland bodies, nuclear indentations, sinusoidal dilatation of nuclear membrane, nucleolar changes like increase in number, its varying size and shape and nucleolar margination, degenerated mitochondria and increased number of ribosomes.<sup>[10,11,20,22]</sup>

It is also evident from this study that some cases of homogenous leukoplakia showing mild dysplasia can show many other alterations under TEM suggesting the need for detailed fine structural evaluation of the precancerous lesions.

The other important finding of the study is that nodular type is the most severe type of leukoplakia, which is the most likely to undergo malignant transformation, as the ultrastructural features of dysplasia were more severe as compare to other types of leukoplakia.

## SUMMARY AND CONCLUSION

One of the greatest challenges to oral oncobiologist is to determine and identify the tissue damage, along with difficulties in identifying the exact transition of normal to precancerous state. In this current scenario with improved immunohistochemical techniques and morphometric analysis, TEM may go hand in hand to provide a better diagnosis and early detection of cancer. Therefore, all the light microscopic and transmission electron microscopic findings were recorded, correlated, analyzed and the following conclusions and assumptions were achieved.

1. Homogenous leukoplakia is characterized by abundant keratinization in the form of thick bundles of tonofibrils and increased keratohyaline granules of varying size ultrastructurally, whereas ulcerative and nodular type showed decreased keratinization observed as decreased number of tonofilaments and few or absence of keratohyaline granules.
2. Homogenous leukoplakia showed intact basement membrane under light microscope but two of them showed discontinuous basal lamina under TEM.
3. Presence of pathologic cytoplasmic processes, which is indicative of neoplastic change, can only be observed under TEM.
4. Disrupted intercellular junctions were seen only in ulcerative and nodular type in light microscopy, whereas under TEM along with this, two types of

homogenous leukoplakia also showed disrupted intercellular junctions, revealing the importance of TEM.

5. Intracellular vacuolization, a light microscopic features is seen as “laking effect” under TEM in most of the cases of leukoplakia.
6. Ulcerative and nodular leukoplakia were characterized by features of decreased keratosis, defects in basal lamina, decreased and ruptured hemidesmosomes, loss of intercellular junctions, severe indentations in the nuclear membrane, sinusoidal dilatation between the nuclear membrane and multiple nucleoli with margination. These ultrastructural features were indicative of severe degree of dysplasia, progressing towards neoplasia.
7. Degenerative mitochondria one of the dysplastic features, which cannot be observed under light microscope, showed cristolysis and fused and disoriented cristae in most of the cases of ulcerative and nodular leukoplakia in all the cell layers.
8. Increased ribosomes, an epithelial dysplastic feature were seen in all the cases. However the rosette formation which is indicative of severe dysplasia was not found in any of the cases of leukoplakia.
9. Odland bodies were observed in most of the cases of oral leukoplakia but could not be related to the epithelial dysplasia.

Thus, it is finally concluded that nodular leukoplakia seems to be the most severe clinical type of leukoplakia showing highest risk of malignant transformation. This severity is detected only by alterations in the fine structural details observed by TEM, which cannot be resolved by light microscope.

This reveals the importance of transmission electron microscope as a useful diagnostic weapon especially for the precancerous lesions.

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