



## **Comparison of Biopsy Samples of Leukoplakia Obtained by Surgical and Laser Excision A Histopathological and Scanning Electron Microscope Study**

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### **ABSTRACT**

*Various types of biopsy techniques are used with each technique having its own advantages and disadvantages. In our study surgical versus laser excision for biopsy of leukoplakia cases and all the samples were analyzed under normal microscope and also under scanning electron microscope to find out whether there was any loss of architecture or charring of mucosa due to laser biopsy. A total of 40 patients (20 in each group) satisfying the inclusion criteria were allocated using surgical and laser groups. The excised samples were sent for histopathological analysis was done under normal microscope and also under scanning electron microscope. Out of 40 participants there was only one female participant. Maximum subjects (33.3%) were in age group of 61-70 years. 20% patients of leukoplakia had a habit of bidi or hukka smoking and very few 2.5% had mixed deleterious habits of chewing tobacco and smoking, or chewing gutka and hukka both. Most common site of biopsy in 40 % population was left buccal mucosa. On obtaining the histopathological report of sample obtained by either surgical or laser excision there was no discrepancy as in both the specimens we saw 55% had mild dysplasia, 22.5% had hyperkeratosis, 12.5% had moderate dysplasia and 5% had mild to moderate dysplastic changes. (p value <0.05) Similarly, when the samples obtained by surgical or laser excision were studied under scanning electron microscope 35 % showed well define intercellular junction and microrugul surface pattern and 65% showed thickened irregular protrusions and evidence of villus like pattern showing dysplasia. (p value <0.05) The results were exactly congruent in both SEM or normal microscopy irrespective of the method of excision of sample whether surgical or laser. Which justifies that In the histological parameters seen under normal microscope as well as SEM, there was no loss of architecture or charring of mucosa or epithelium in the laser group. Both biopsy techniques seem to be equally effective in performing excisional biopsies of oral lesions. On studying the specimen under normal as well as under SEM Laser has many advantages of being noninvasive, bloodless field and no suturing as well and according to present study LASER excision was not causing any loss of histological architecture at all making it a better choice of biopsy in future*

**Keywords:** Excisional biopsy, Histopathology, Laser biopsy, Scalpel biopsy, Scanning electron microscope (SEM)

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### **INTRODUCTION**

The term leukoplakia should be used to recognize white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer [1]. The main factors that make a individual more vulnerable for the development of leukoplakia are deleterious habit especially smoking and alcohol consumption, sharp tooth cusp, any physical irritants, electronic current, chronic trauma, poor oral hygiene and micro-organisms such as fungus like candida albicans and virus/ human papilloma virus (HPV). Researchers estimated malignant transformation rate of leukoplakia is 5% to 6%. In any case, the clinical appearance of a leukoplakic lesion is not directly indicative of the histology [2, 3]. The management of leukoplakia includes multiple factors like discontinuation of deleterious habits, diet having good nutritive value, vitamin supplements, antifungal drugs and few chemotherapeutic agents and surgical management modalities have been widely used with vivid results. If the lesion is small, excisional biopsy is recommended though it is advisable to perform incisional biopsy of larger lesions for histopathological examination that will guide us with better treatment protocols that can be followed Other than this depending on the size and site of lesion cryosurgery and complete laser ablation can be done. In Diode laser the wavelength range is about 810-980 nm [4]. The ability to use this instrument in a wave or pulsed mode increases its utility in soft tissue surgery. This laser has high absorption in soft tissues with pigmentation such as hemoglobin, melanin and collagen chromophores

and low absorption in dental hard tissues. It is indicated for surgery of oral soft tissues close to dental structures that does not involve excessive bleeding [5-7]. In present study we are evaluating the efficacy of lasers for excisional or incisional biopsies and comparing it with surgical or scalpel biopsies at a histopathological level by examining the procured biopsy specimen under normal as well as scanning electron microscope so that we will clearly see under SEM whether there was any loss of histological structure at tissue or cellular levels because of thermal damage caused by usage of LASERS. Hence to depict whether the Lasers can be used with same histopathological efficacy as surgical/ scalpel for taking biopsy we had to formulate a study by altering the methodology of taking the biopsy specimen by usage of lasers in this as discussed in detail we included the normal tissue area of approximately 1cm and also a low wavelength of 650 nm was used to produce minimum heat or thermal damage to effected tissue site with leukoplakic changes [13-20]. Also while taking the biopsy specimen we tried to include the biopsy site from part of mucosa which was having a larger lesion .

## MATERIALS AND METHODS

We conducted this study on patients visiting the OPD of dept. of oral medicine and radiology of SGT dental college and hospital. A proper ethical clearance was obtained before performing the study and the ethical clearance letter was procured from institutional ethical committee. The aim of the study was to compare the efficacy lasers versus scalpel/ surgical aid in performing biopsy of oral lesions of leukoplakia . In this study we evaluated the healing process after scalpel and laser biopsy, to evaluate patient comfort during laser and scalpel surgery, and to most importantly analyze the histological and scanning electron microscope parameters of the biopsy specimen obtained with each of these techniques.. Appropriate patients consent was taking in his own language for including the participant for the study. Patients were made to sit comfortably on a dental chair. Recording of general history, history of deleterious habits, clinical examination was done in a systematic manner and detailed clinical examination of lesion was done and entered in the case performa specially designed for the study .Diagnosis and clinical examination of leukoplakia was made based on clinical examination and history followed by histopathological assessment under normal and scanning electron microscope Patients with any other lesion in the affected site with oral lichen planus and OSMF, or having anticoagulant, systemic steroids, immunosuppressive drugs, any history of carcinoma, immunodeficiency/HIV were excluded from the study group. ZOLAR diode laser (980nm,3watt, contact mode) was used for LASER The study was consisted of randomly selected 40 samples of patients diagnosed as oral leukoplakia, the study sample who were enrolled for the study were within the age group of 30- 70 years. Laser fiberoptic tips of 320 micro meter were used ,in pulsating mode ,duration of application was approximating 10-12 seconds per site ,and duration varied according to the size of the lesion. Lesion was irradiated by diode laser of 6 watts ,till area changed to white (photocoagulation).. Group I included biopsy taken from oral lesions with scalpel. Group II included biopsy taken from oral lesions with diode laser. Twenty patients underwent biopsy using scalpel. In 20 patients, excision of the lesion was done with 980nm diode laser. For all the lesions, a 0.5 mm of safety margin was kept in both the groups . The excised samples were sent for histopathological and scanning electron microscope analysis were recorded. The data collected were tabulated and statistically analyzed. Keeping the  $p < 0.05$  as significant the appropriate statistical tools were used to compare the observations.

## RESULTS

The study was conducted in Department of Oral Medicine and Radiology, SGT Dental College and Hospital, Gurugram ,Data collected was subjected to statistical analysis using IBM Short Package for the Social Science 20.0 software. Descriptive and comparative analysis was done.

**Table no. 1: Mean age and Gender wise distribution**

Statistics			
	Male	Female	Total
N	39	1	40
Mean	52.9143	54.0000	52.9444
Median	50.0000	54.0000	52.0000
Mode	38.00 <sup>a</sup>	54.00	38.00 <sup>a</sup>
Std. Deviation	15.74497		15.51947
Variance	247.904		240.854
Range	57.00	.00	57.00
Minimum	28.00	54.00	28.00
Maximum	70.00	54.00	70.00

a. Multiple modes exist. The smallest value is shown

**Table no. 1** shows Mean age and Gender wise distribution. Out of 40 subjects, 39 were males and 1 was female, with mean age 52.91yrs and 54yrs respectively. Chi square statistical analysis revealed an insignificant level (p-value>0.05) of significance statistically.

**Table no. 2: Age wise distribution**

Age groups	No. of subjects	Percentage
<20	0	0
21-30	3	5.555556
31-40	10	22.22222
41-50	9	22.22222
51-60	6	16.66667
61-70	12	33.33
Total	40	100
Chi square	5.998	

\*p-value<0.05 is significant.

**Table no. 2** shows Age wise distribution. Maximum subjects were 33.33% in 61-70yrs, followed by (22.22%) were of age group 31-40yrs and 41-50yrs. Minimum subjects aged 21-30, and 31-40 of age. Chi square statistical analysis revealed an insignificant level (p-value>0.05) of significance statistically.

**Table no. 3: Habit wise distribution**

Habits	No. of subjects	Percentage%
Biddi	8	20
Gutka Chewing	4	10
Consume Gutka	2	5
Gutka Chewing & Biddi Smoking	2	5
Consume Hukka	8	20
Consumes Tobacco	1	2.5
Gutka Chewing & Hukka	1	2.5
Hukka	4	10
Smokes Biddi & Hukka	2	5
Smokes Cigarette	7	17.5
Smokes Cigarette & Hukka	1	2.5
Total	40	100

**Table no. 3** shows habit wise distribution. Maximum number of patients, 20% each were biddi and hukka consumers, followed by 17.5% cigarette smoker. Minimum number of patients, 2.5% consume tobacco, gutka and hukka both, and smoke cigarette and hukka both.

**Table no. 4: Site wise distribution**

Site	No. of subjects	Percentage
Left & Right Buccal Mucosa	2	5
Left & Right Commissural +of Lip	3	7.5
Left Buccal Mucosa	16	40
Right Buccal Mucosa	14	35
Left Commissural of Lip	3	7.5
Right Buccal Mucosa, Commissural of lip	1	2.5
Right Retro Commissural area	1	2.5
Total	40	100
Chi square	10.753	
p-value	0.014*	

\*p-value<0.05 is significant.

**Table no. 4** shows **Site wise distribution**. Maximum number of patients, 40% had lesion on left buccal mucosa, followed by 35% on right buccal mucosa, followed by 7.5% on left commissural of lip and both right-left commissurals of lip. Minimum number of patients, 2.5% had lesion on both right buccal mucosa and commissural of lip, and right retro commissural area.

**Table no. 5: Histopathological features of leukoplakia samples obtained by surgical biopsy excision**

FEATURES	No. of subjects	Percentage
Mild Dysplasia	22	55
Moderate Dysplasia	7	17.5
Severe Dysplasia	2	5
Hyperkerotosis	9	22.5
<b>Total</b>	40	100
Chi square	8.023	
p-value	0.052*	

\*p-value<0.05 is significant.

**Table no. 5** shows Histopathological features of leukoplakia by surgical biopsy under normal microscope. Maximum number of patients, 55% shows mild dysplasia; 22.5% shows hyperkeratosis; 12.5% shows moderate dysplasia and minimum 5% showed mild to moderate dysplasia and severe dysplasia. Chi square statistical analysis shows a significant difference statistically (p-value<0.05).

**Table no. 6: Scanning electron microscope features of leukoplakia samples obtained by laser biopsy**

FEATURES	No. of subjects	Percentage
Thickened irregular protusions & evidence of villus like pattern showing Dysplasia	14	35
Well defined intercellular junction & the microrugal surface pattern	26	65
<b>Total</b>	40	100
Chi square	12.799	
p-value	0.052*	

\*p-value<0.05 is significant.

**Table no. 6** shows Histopathological features of leukoplakia by laser biopsy under scanning electron microscope. 35% shows Well defined intercellular junction & the microrugal surface pattern; and 65% shows Thickened irregular protusions& evidence of villus like pattern showing Dysplasia. Chi square statistical analysis shows a significant difference statistically (p-value<0.05).

**Table no. 7: Histopathological features of leukoplakia samples obtained by laser excision**

FEATURES	No. of subjects	Percentage
Mild Dysplasia	22	55
Moderate Dysplasia	7	17.5
Severe Dysplasia	2	5
Hyperkerotosis	9	22.5
<b>Total</b>	40	100
Chi square	8.023	
p-value	0.052*	

\*p-value<0.05 is significant.

**Table no. 7** shows Histopathological features of leukoplakia by laser biopsy in under normal microscope. Maximum number of patients, 55% shows mild dysplasia; 22.5% shows hyperkeratosis; 12.5% shows moderate dysplasia and minimum 5% shows mild to moderate dysplasia and severe dysplasia. Chi square statistical analysis shows a significant difference statistically (p-value<0.05).

**Table no. 8: Scanning electron microscope features of leukoplakia samples obtained by surgical excision**

FEATURES	No. of subjects	Percentage
Increase number of RBC'S , WBC'S and Fibroblasts with thickened irregular protusion& micro rugal patterns	15	37.5
RBC'S, WBC'S, Fibroblasts seen with well defined intercellular junction & villus like pattern	25	62.5
<b>Total</b>	40	100
Chi square	7.722	
p-value	0.055*	

\*p-value<0.05 is significant.

**Table no. 9** shows Comparison of Histopathological features of leukoplakia by surgical and laser biopsy under scanning electron microscope. In surgical and laser group, histopathological features are different under SEM, with a significant difference ( $p$ -value $<0.05$ ) statistically.

## DISCUSSION

Although, there has been a great amount of development of newer diagnostic methods for diagnosis of oral cancer and precancer, biopsy and histopathological examination are still considered to be the gold standard. Three methods of oral soft tissues biopsies are used commonly in dentistry, namely scalpel, electrocautery and laser.(8) Morphological and functional recovery of oral tissues following diode 980 nm laser oral biopsy is superior when compared with conventional scalpel oral biopsy. In present study we decided to utilize a 980 nm diode laser for our patients because of its availability, convenience of use, bloodless field, capacity to treat vast regions in a single application, and the ability to precisely regulate laser fluency in all parts of the mouth. The following adjusted parameters were used in laser excision: 0.5 mm of normal mucosa was added as a safety margin at the perimeter of the leukoplakic lesion to minimise charring and thermal damage of the mucosa by laser biopsy. Low-power settings using a diode laser at 980nm and 3 watts were used to remove the lesion until the region turned white. In present study, maximum number of patients, 20% each were bidhi & hukka consumers, followed by 17.5% cigarette smoker. Minimum number of patients, 2.5% consumes tobacco, gutka & hukka both, & smoke cigarette & hukka both as shown in Table & graph 3. The most common sites of leukoplakia invasion are buccal mucosa, floor of the mouth, retrocommissural areas, border & sulcus of the tongue. In present study, we found that Maximum number of patients, 40% were involving left buccal mucosa, followed by 35% right buccal mucosa, followed by 7.5% left commissural of lip & both right-left commissural of lip. Minimum number of patients, 2.5% involve both right buccal mucosa & commissural of lip, & right retro commissural areas shown in Table 4 & Graph 4. In accordance with our study, Kumar et al found that the most commonly affected site was buccal mucosa.Kharadi UA et al, also advocated the use of 980nm diode laser, as safe can be used for diagnosis as well as treatment modality of leukoplakia, without any complication & without compromising health & oral function of patients(8). Thus, we compared the efficacy of laser & scalpel in taking biopsy & further compared the efficiency of histopathological features observed under normal microscope & SEM.” We observed dysplastic changes in specimens excised either with scalpel or diode laser using histopathology. It has been observed that histopathological features of leukoplakia by surgical biopsy under normal microscope, 55% showed mild dysplasia; 22.5% showed hyperkeratosis; 17.5% showed moderate dysplasia & minimum 5% showed severe dysplasia, with a significant difference statistically ( $p$ -value $<0.05$ ). as shown in Table & Graph 5) Yasmeen SA et al, stated that diode laser is a definitive technique with marked clinical improvement with high degree of patient acceptance in comparison to scalpel excision [9]. The excisional biopsy with Bard-Parker blade is the most common way of management of premalignant or malignant lesions. However, postoperative hemorrhage is a common complication associated with the surgical procedures of the tongue (10). Alternative methods that can do away with blades, sutures, & bleeding, are likely to be more acceptable by patients. Thus the authors found that excisional biopsy of lesion done with 940- nm diode laser is a safe, painless bloodless diagnostic & surgical treatment option. Deepa et al stated that when the excisional biopsy was done using diode laser at a low power setting of 2.5 watt in contact mode. Diode lasers proved to be effective in histopathological diagnosis and treatment of oral leukoplakic lesion. In our study scanning electron microscope features of leukoplakia samples obtained by laser excision 65% shows well defined intercellular junction & micro rugal patterns, 35% showing Thickened irregular protusions& evidence of villus like pattern showing dysplasia with a significant difference statistically ( $p$ -value $<0.05$ ). As shown in Table 6 In our study histopathological feature of leukoplakia samples obtained by laser excision shows, Maximum number of patients, 55% shows mild dysplasia; 22.5% shows hyperkeratosis; 17.5% shows moderate dysplasia & 5% shows severe dysplasia, with a significant difference statistically ( $p$ -value $<0.05$ ) as shown in Table 7 Scanning Electron Microscope features of leukoplakia sample obtained by surgical excision were also observed under, 62.5% shows RBC'S, WBC'S, Fibroblasts seen with well-defined intercellular junction & micro rugal patterns, followed by 37.5% shows RBC'S, WBC'S, Fibroblasts seen with thickened irregular protusion villus like pattern with a significant difference statistically ( $p$ -value $<0.05$ ) (As shown in Table 8). When comparison of histopathology of biopsy samples of leukoplakia obtain by surgical excision & laser excision was done it shows both surgical & laser biopsy shows similar results under normal microscope as shown in (Table 9). Thus Chi square statistical analysis shows a significant difference statistically ( $p$ -value $<0.05$ ). Which suggests that due to change in methodology where we involve 0.5mm of normal mucosa as safety margins & low power settings with help of diode laser 980nm & 3 watt, there was no tissue charring that may lead to discrepancy in

histopathological features by surgical or laser biopsy. There was loss of minute architectural details at subcellular level such as RBC'S, WBC'S, and fibroblasts in laser biopsy samples which could be due to thermocoagulation. These results are congruent with Khare et al their study also showed that both the techniques are equally effective in performing excisional biopsies with laser biopsy of oral lesions [8]. They used 970nm diode/ NdYg laser with involvement of 0.5 mm safety margins According to them Laser has the advantage of maintaining a bloodless field & avoidance of suturing as well. However, due to the associated thermal damage caused, there may be minor loss of histological architecture. Comparison of histopathology & scanning electron microscope features of biopsy samples of leukoplakia obtained by laser excision as shown in table no. 10 & Graph no. 10 shows. In laser biopsy shows 12.5% mild dysplasia, moderate 62.5%, severe 2.5% & hyperkeratosis 22.5%. Also, In surgical biopsy shows 55% mild dysplasia, moderate dysplasia 17.5%, severe dysplasia 5% & hyperkeratosis 22.57% results under normal microscope, Thus result shows with a significant difference (p-value<0.05) statistically. Hence when compared the histological features of samples taking by laser biopsy showed more number of moderate dysplasia (62.6%). As compare to surgical biopsy 17.5% moderate dysplasia (p value <0.05) which indicates that laser can be used as a highly effective tool for obtaining biopsy samples without compromising on the histopathological accuracy/diagnosis of leukoplakic lesions. These results are congruent with previous studies done by Khare et al using diode lasers [11]. Comparison of histopathology & scanning electron microscope features of biopsy samples of leukoplakia obtained by surgical excision & laser excision. In surgical biopsy shows 55% mild dysplasia, moderate dysplasia 17.5%, severe dysplasia 5% & hyperkeratosis 22.57% results under normal microscope, Also in laser biopsy shoes 12.5% mild dysplasia, moderate 62.5%, severe 2.5% & hyperkeratosis 22.5% Thus result shows with a significant difference (p-value<0.05) (Table 8). Comparison of scanning electron microscope findings of biopsy samples obtained by surgical excision & laser excision (Table 9). In surgical & laser group, histopathological features are different under SEM, with a significant difference (p-value<0.05) statistically. Hence, samples studied by scanning electron microscope shows more number of moderate dysplastic changes 62.5% in scanning electron microscope & 17.5% in normal microscope which clearly indicates that scanning electron microscope has better efficacy in detecting even the minutest of epithelial dysplastic changes at cellular & subcellular levels, hence clearly indicates that scanning electron microscope can be a better detecting tool of dysplastic changes, this features of scanning electron microscope can help in early detection of dysplasia as well as conversion of leukoplakic lesion to squamous cell carcinoma. No previous studies are available having similar methodology, result or observation in literature. Our study is first in its kind to compare entities like laser & SEM with conventional surgical biopsy & histopathology. Many more studies with increased sample size should be done in order to prove the accurate efficacy & evaluation of use of lasers as well as SEM for deciding the diagnosis as well as prognosis of premalignant disorders such as leucoplakia [9-19]. Hence we conclude that because of comfort level of use of lasers for the operator & the patient's benefit which includes fast healing, painless invasion, bloodless field, diode laser can be recommended in taking biopsy of leukoplakia lesions.

## CONCLUSION:

We conclude that due to change in methodology where we involve 0.5mm of normal mucosa as safety margins and low power settings with help of diode laser 980nm and 3 watt, there was no tissue charring that may lead to no discrepancy in histopathological features by surgical or laser biopsy this was thoroughly analyzed and evaluated by normal microscopic examination as well Scanning electron microscope (SEM) to rule out any changes at tissue, cellular, subcellular levels like organelles, mitochondria, ribosomes, desmosomes, RBC etc. This detailed histopathological examination of biopsy tissue taken by surgical or laser biopsy truly revealed that LASER are a future potential tool to take biopsy as they are less invasive, bloodless and cause less discomfort to patients but in future more studies with larger sample size can make it a gold standard biopsy procedure for which researchers have to modify their methodology for taking the biopsy specimen through LASER tools to avoid loss of tissue architecture which was done and followed in our present study.

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