EVALUATION OF EFFICACY OF LASER ABLATION IN THE MANAGEMENT OF ORAL LEUKOPLAKIA

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INTRODUCTION

Leukoplakia is one of the most common premalignant lesions of oral cavity. WHO defined leukoplakia as a lesion which has a white patch or plaque on the oral mucosa, that cannot be removed by scraping and cannot be classified clinically or histopathologically as any another disease activity (WHO1978). Oral leukoplakia has been redefined as: A predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion, some oral leukoplakia will transform into cancer(Axell T, 1996)

Definitive treatment of oral leukoplakia is very important because of its recurrence and malignant transformation. There are different treatment methods for oral leukoplakia. They are -

1) Surgical excision with or without grafting,
2) Cryosurgery,
3) Laser ablation,
4) Anti-fungal therapy - in case of Candida associated leukoplakia,
5) Chemoprevention[1]

Surgical excision is commonly employed because of its ease of use, accuracy, minimal damage to tissue and low cost. However, it do not provide a good hemostasis which is important on highly perfused tissues such as in the oral cavity.[2] There may be high chances of recurrence and infection wound contraction and scarring.[3]

Laser surgery for oral mucosal lesion has been reported to have many advantage and it is widely used in the treatment of oral leukoplakia.[1] More recently, the use of

diode laser surgery has been recommended. Diode laser is used for soft tissue procedure and wavelength is well absorbed by soft tissue, providing good hemostasis and effective cutting of the tissue.[5]

Specific advantage of laser surgery include rapid and precise tissue dissection, control of bleeding because of sealing of blood and lymphatic vessel, low postoperative pain, low morbidity, reduced tissue scarring and wound contraction, Excellent patient acceptance and faster healing.[4]

Vitamin A in the form of retinoids, isotretinoin, beta carotene, 13 cRA, 4 HPR are used in many clinical trials of oral leukoplakia and the use of topical and systemic retinoids is most commonly used antioxidant in treatment of oral potentially malignant disorders like leukoplakia,. 13-cRA though has been shown to be effective in resolving oral leukoplakia but high rate of recurrence after treatment and adverse effects are reported in some studies.[6]

Based on the above points it is worth that a study can be carried out by comparing the two modalities. Hence we intended to compare the efficiency of diode laser ablation and conventional antoxidants in treating patients with oral leukoplakia.

Antioxidants play an important role in scavenging toxic free radicals in the tumor sites. This helps in preventing progression of an established carcinoma or in checking the malignant transformation of the cells which are exposed to the harmful effects of free radicals.[7]

Oxidative stress occurs when there is an imbalance between the production of Reactive Oxygen Species and a cell's oxidant capacity or when there is a decrease in this capacity.This stress may cause mutagenesis, cytotoxicity and changes in gene expression

that initiate or promote carcinogenesis(10-ref11-11).Polyunsaturated fatty acids (PUFA) are the major class of biomolecules susceptible to oxidative damage by ROS; this process is referred to as lipid peroxidation[7,8]

In this system, superoxide dismutase(SOD) convert superoxide radical (O2 •_) into H2O2, whereas glutathione peroxidase (GPx) and catalase convert H2O2 into water(8,9). Therefore, two toxic species, O2 •_ and H2O2, are converted into the harmless product water.[7,8] Apart from analyzing the clinical efficacy of the two treatment modalities we have implicated the assessment of two biomarkers in saliva which are SOD(Superoxide dismutase) and GSH(Glutathione).The levels of these two antoxidants of saliva are measured preoperatively and post operatively and compared.

AIMS AND OBJECTIVES

AIM OF THE STUDY

Aim of the study is to evaluate the efficacy of Diode LASER ablation in the treatment of oral leukoplakia.

OBJECTIVES

1. Use of Diode LASER for treating oral leukoplakia.
2. Compare it with Antioxidant therapy.
3. Three months clinical follow up of patients in each treatment group.
4. Evaluation of salivary antioxidants(SOD and GSH) in the pre-treatment period and after three months follow up.

REVIEW OF LITERATURE

TREATMENT OF PATIENTS WITH ANTIOXIDANTS

Oral leukoplakia is considered as a potentially malignant condition with a fare rate of malignant transformation potential. Cellular dysplasia found in the histopathology of leukoplakia lesions is due to the adverse effects of lipid peroxidation and the formation of reactive oxygen species. The treatment of leukoplakia lesions with antioxidants was introduced with an aim to reduce this oxidative stress. Vitamin A, Vitamin C and Vitamin D are used in various forms as antioxidant supply for the treatment of patients with oral leukoplakia.[9]

Vitamin A in the form of retinoids, isotretinoin, beta carotene, 13 cRA, 4 HPR are used in many clinical trials of oral leukoplakia and the use of topical and systemic retinoids is most commonly used antioxidant in treatment of oral potentially malignant disorders like leukoplakia and lichen planus. 13-cRA though has been shown to be effective in resolving oral leukoplakia but high rate of recurrence after treatment and adverse effects are reported in some studies . Beta carotene is the precursor of vitamin A and is commonly seen in the yellow colour fruits and vegetables like carrot, sweet potato, mango, papaya, oranges and is been reported to be most effective in leukoplakia associated with smokers and it's therapeutic effect is due to its linkage with oxygen which is an unstable reactive molecule, thus diminishing the damaging effects of free radicals . Fenretinide ability to inhibit cell growth through the induction of apoptosis has proven to be less toxic than many other vitamin A analogues. [10]

Shah et al in 1983 conducted a study in 11 patients using topical vitamin A 1 -5 mg.The duration of study was 11 months.He observed 27% clinical resolution of the lesion and 18 % recurrence over the study period.Malignant transformation rate is not reported in the study. No adverse effect of the antioxidant therapy was reported.[11]

Hong et al in 1986 did a study on leukoplakia patients using 13-cis -retinoic acid 1 to 2 mg per kilogram of body weight per day for 3 months .The sample size taken was 44 in which actual treatment was done for 24 patients and 20 patients taken as placebo group.Follow up period of the study was 9 months.Clinical resolution noticed in 67 % patient versus 10% in placebo group. Reversal of dysplasia occured in 54% of the patient versus 10 % of placebo group.Relapse of the clinical lesion was noticed in 9 patients.Adverse drug effect like cheilitis, facial erythema and dryness,peeling of skin,conjunctivitis and hypertriglyceridemia was noticed in 2 patients.[12]

Stich et al 1988 in their study conducted in 65 leukoplakia patients(30 vitamin A 35 placebo over a period of 6 months) ,57% complete remission was observed in vitamin A group.21% of patients in placebo group got new oral leukoplakia lesion where as no new lesion developed in vitamin A group.Dosage of antioxidant used was 14 mg per kg body weight per day .No malignant transformation and adverse drug effects were reported.[13]

Stich et al in 1988 : Another study was conducted by the same author, for 6 months duration. The study group was divided into 3,group 1(beta-carotene 180 mg per week) ,group 2 (carotene 180 mg per week and vitamin A 100000 IU per week) and group 3 (placebo). Remission of OL in group 1 (14.8%) and group 2 (27.5%) differed

significantly from that seen in group 3(3%).Adverse effects of the drug were not reported.[14]

Garewal et al in 1990 ,in his study, noticed 8 % clinical resolution and 8 % malignant transformation in 24 leukoplakia patients treated with systemic beta-carotene 30 mg per day for a period of 6 months.Recurrence of resolved lesions is not mentioned in the study .Adverse effect of drugs were also not noticed.[15]

Toma et al in 1992 in a similar study using systemic beta-carotene 90 mg per day in 23 patients.26% clinical resolution and 5 % recurrence were the main findings in the 7 month study . Malignant transformation was not reported in the study.The adverse drug effect of antioxidant used in the study were also absent.[16]

Toma et al in 1992 again conducted a study using 13-cis- retinoic acid in 16 leukoplakia patients over a period of 6 months. The initial dose, given for 3 months, was 0.2 mg/kg/day, increasing by further 0.2 mg/kg/day in. successive 3 month cycles. The maximum dosage reached was 1.0mg/kg/day. Fourteen of the patients completed the trial and there was one complete response obtained at 0.4 mg/kg/day. After the retinoic acid treatment was stopped, patients were followed-up for 12 months; 2 patients showed regression of the initial responses after 6 and 9 months.None of the patients showed adverse effect of the therapy.[17]

S.M. Lipman et al 1993 in a three phase study used isotretinoin 1.5 mg per kg per day in 65 patients with oral lekoplakia for 3 months.In the first phase,Beta-carotene 30 mg per day was the treatment option in phase 2 for 59 patients.After phase 2 treatment 26 patients were randomly selected for phase 3 tratment using isotretinoin 5 mg per kg per

day. Twenty-two patients (92%) with isotretinoin and 13 patients (45%) with beta-carotene demonstrated a positive response.Drug adverse effects were not reported in the study.[18]

Tradati et al in 1994 conducted a study in 8 oral leukoplakia patient using topical fenretinide(4-HPR).The Duration of study and dosage of the drug used were not reported.The author observed 25 % clinical resolution in his patients. Malignant transformation, recurrence and adverse drug effects were also not reported.[19]

I.W.Dimery et al in 1997 ,in his study in 7 patirnts with oral leukoplakia using 13-cis-retionoic acid in an escalating dose from 800 IU per day to a maximum of 2000 IU per day for 4 months noticed 71% complete resolution of the lesions.No other relevant data was reported in his study.[20]

Sankaranarayanan et al 1997 conducted a double-blind placebo-controlled trial to evaluate the chemopreventive potential of either vitamin A alone or beta carotene alone in subjects with oral leukoplakia in Kerala, 110 patients divided into 55 treatment group and 55 placebo group were included in the study .The time period of the study using systemic beta -carotene 360 mg per week was 12 months.54% clinical resolution and 5 % recurrence were observed .Malignant transformation was not reported in the study. head ache and muscular pain were the advers effects of the drugs used.[21]

Sankaranarayanan et al 1997 in a smilar study, another antioxidant, systemic isotretinoin 300000 IU per week, found 52 % clinical resolution 67 % recurrence without any malignant transformation. The study group consisted of 105 patients which was

divided into 50 treatment group and 55 placebo group for 12 months.Here the adverse effects of the drug used were head ache ,muscular pain and dry mouth.[21]

Liede et al 1998 conducted a study in 24 patients with oral leukoplakia for 60 to 84 months including follow up by using systemic beta-carotene 20 mg per day. No statistically significant results regarding clinical resolution, malignant transformation, recurrence and adverse drug effects were reported in the study.[22]

Garewal et al 1999 in a two phase study, used systemic beta-carotene 60 mg per day in phase 1 and beta-carotene or placebo in phase 2.50 patients of 21 males and 29 females over a period of 6 months for phase 1 and 6 months for phase 2 were included in the study design.Phase 1 showed 52 % clinical resolution of lesions.In contrast phase 2 showed 18% recurrence in the group treated with beta- carotene and 17 % in placebo group. Notably there was 38% of malignant transformation noticed in phase 2.Adverse effects of systemic therapy were not mentioned in the study. [23]

Joel B. Epstein & Meir Gorsky 1999 in their case series of 26 patients (17 men and 9 women) with oral leukoplakia using topical .05 % vitamin A(tretinon) acid gel 4 times a day, noticed complete response in 3 patients, partial response in 14 patients, no response in 4 patients and recurrence in 3 patients.Lichenoid reaction in 35% of patients and sensitivity in 19 % of patients were the adverse effects of the topical treatment. [24]

Piattelli et al 1999 in 10 oral leukoplakia patients which were divided into 5 treatment group and 5 placebo group tried topical isotretinoin 1% as the treatment option.Time period of the study was 48 months .10% clinical resolution was observed.

There were no reports on malignant transformation, recurrence and adverse drug effects.[25]

Jack Lee et al 2000 in their study in 70 patients which was done in 2 phases: phase1:isotretinoin (1.5 mg/kg ,phase 2: low dose isotretinoin (0.5 mg/kg/day) or β-carotene (30 mg/day) found malignant transformation in 22 of 70 patients(31.4%) ,most of them were in low dose isotretinoin and beta-carotene group, adverse effects of the systemic antioxidant drugs were not mentioned in the study.[26]

Gaeta et al 2000 did treatment in 21 leukoplakia patients with 14 patients in treatment group and 7 patients in placebo group using topical acitretin .The resolution, recurrence and malignant transformation were not mention in the study. But they could find no adverse effects of the drug used. [27]

Beenken et al 2000 in his study used Oral Fenretinide given in daily dosage for 3 months was the treatment option in the study by.The number of patients with oral leukoplakia in the study were 30.Results and follow up of the study was not published. [28]

F .Femiano et al 2001 treated20 patients with calcipotriol 50 mg per gram ang 20 patients with tretinoin cream .04 %for 5 weeks.16 patients in both groups had complete resolution. Recurrence and malignant transformation was not reported.No topical and systemic adverse effects was reported in 4 months follow up. [29]

Fausto Chiesa et al 2005 in his study of 170 patients (121 males and 49 females) used fenretinide(4-HPR) 200 mg per day(100 mg thrice a day) to treat 84 patients for 12 months .86 patients kept in the control group. 43 completed treatment at full dosage with 90% compliance. 15 recurrence and 10 new lesions in control and 15 recurrence and 4

new lesions in cases treated using 4-HPR were noticed. Malignant transformation was reported in both groups. 9 out of 43 had mild adverse effects 14 with major effects: hematologic toxicity in 7, cutaneous toxicity in 6 and gastric toxicity in one. [30]

Lippman et al 2006 in his 9 months study,Fenretinide(4-HPR) 200 mg per day was also tried in 35 oral leukoplakia patients .0%clinical resolution and 23% malignant transformation were the highlighted results of the study. Drug adverse effects were not reported in the study. [31]

G.A.Scardina et al 2006 did comparison of two topical treatment using isotretinoin 0.18% and 0.05 % twice a day for 3 months No topical and systemic adverse reaction were reported in 10 year follow up. Clinical resolution was found 85% in 0.18% group. [32]

ROLE OF OTHER ANTIOXIDANTS IN TREATMENT OF ORAL LEUKOPLAKIA

Vitamin C [L- ascorbic acid],Vitamin E [alpha tocoferol] and lycopene are the other oxidants tried in few clinical trials when compared to vitamin A. Lycopene is the natural pigment synthesised by plants and microorganism and is present in tomatoes, water melon, guavas, grapefruits, red chillies etc., Lycopene appears to be a very promising antioxidant as a treatment modality in oral leukoplakia and can protect cells against cell damage and play a protective role against progression of dysplasia by inhibiting tumour cell proliferation and the first report of efficacy of lycopene against human oral cancer cell was published describing the significant therapeutic effect.

Benner et al 1993 conducted a study in 45 patients in a duration of 23 months using systemic Alpha Tocopherol 400 IU.20 % resolution was noticed in the study.Recurrence ,malignant transformation and adverse effects of the drug were not reported in the study. [33]

G.E .Kaugars et al 1994 treated 79 patients using 30 mg of beta-carotene, 1000 mg of L-AA [L-ascorbic acid] and 800 IU of AT [alpha tocoferol] per day.the duration of the study was 9 months. 55.7% showed reduction in the size. Clinical improvement was observed in 90% of the patients who had reduced risk factors, compared with 48.8% of improvement in those who did not. Squamous cell carcinoma developed in seven patients (8.9%)Drug adverse effects were not reported. [34]

T.J.Barth et al 1997 used beta-carotene vitamin Eand L-AA [L-ascorbic acid] to treat with 25 patients with oral leukoplakia. In 97.5% of patients, dysplasias were

diminished by use of antioxidant combinations and is more evident in patients with cessation of habit without any drug adverse effects. [35]

Singh et al 2004 in his study, systemic lycopene was the treatment option used to treat 58 patients divided into 3 groups .Group 1 consisted of 20 patients who were treated with 4 mg systemic lycopene.Group 2 also consisted of 20 patients who were treated with 8 mg of lycopene.Group 3 was the placebo group consisted of 18 patients.Duration of the study was 3 months. 80%, 66.25% and 12.5% clinical resolution in 3 groups respectively. Recurrence & Malignant transformation and adverse drug effects were not reported. [36]

Win Pa Pa Aung et al in their 5 oral leukoplakia used 500 mg lycopene twice a day for 3 months .Mild improvement in thin leukoplakia lesions were noticed .The adverse effects of systemic lycopene therapy were not reported in the study.[37]

LASER

HISTORY:

Einstein in 1917 gave the first theoretical foundation of LASER and MASER using Plank's law of radiation that was based on probability coefficients (Einstein coefficients) for absorption and spontaneous and stimulated emission of electromagnetic radiation.[38] Theodore Maiman was the first person to demonstrate the initial practical laser in 1960 after the reports by numerous scientists, including R.W. Ladenburg gave the first theoretical description on stimulated emission and negative absorption in 1928 and its experimental demonstration given by W.C. Lamb and R.C. Rutherford in 1947 and the proposal of Alfred Kastler on optical pumping in 1950 and its demonstration by Brossel, Kastler, and Winter two years later. Maiman's first laser was based on optical pumping of synthetic ruby crystal using a flash lamp that generated pulsed red laser radiation at 694 nm. Iranian scientists Javan and Bennett made the first gas laser using a mixture of He and Ne gases in the ratio of 1: 10 in the 1960. R. N. Hall demonstrated the first diode laser made of gallium arsenide (GaAs) in 1962, which emitted radiation at 850 nm, and later in the same year Nick Holonyak developed the first semiconductor visible-light emitting laser.[39]

LASER DELIVERY SYSTEMS:

The existing range of laser delivery systems includes the following:

1. Articulated arms (with mirrors at joints) – for UV, visible, and infrared lasers
2. Hollow waveguides (flexible tube with reflecting internal surfaces) – for middle and far infrared lasers
3. Fiber optics – for visible and near infrared lasers.

LASER EMISSION MODES:

The monochromatic (light of one specific wavelength), directional (low divergence), and coherent (all waves are in a certain phase relationship to each other) Laser lights can be delivered onto target tissue as a continuous wave, gated-pulse mode, or free running pulse mode. In continuous wave mode, the beam is emitted at one power level continuously as long as the foot switch is pressed. In gated-pulse mode, the laser is in an on and off mode at periods. The duration of the on and off timer is in micro seconds. In free running pulse mode, very large laser energy is emitted for an extremely short span in microseconds, followed by a relatively long time at which the laser is off. [38,40]

BASIC DESIGN OF A DIODE LASER:

Main advantage of diode lasers is their size which is apparent to the naked eye. The development of laser emitting micro-structure diode cells reduced the bulk of laser systems to a great extent. The latest dental diode lasers have been designed with dimensions imitating a standard phone. Solid material active media (e.g. GaAlAs – Gallium Aluminum Arsenide) alone is used in diode lasers. Because of the crystalline nature of the active medium, the ends of the crystal can be selectively polished relative to internal refractive indices to produce totally and partially reflective surfaces thus serving the same function as the optical resonators of larger laser systems. The discharge of current across the active medium releases photons from the active medium, finally resulting in the generation of laser light of a specific wavelength, which is determined by the active medium used. [41]

At present, each diode "chip" produces relatively low-energy output. Low power diode lasers operates in milliwatt range, are generally being advertised for low level laser therapy (LLLT). To achieve the power necessary for various dental procedures (e.g. soft tissue surgery), present dental diode lasers uses the banks of individual diode chips in parallel to obtain the appropriate power levels (several watts). Some dental diode lasers can also be set to lower power (milliwatt range) and can perform LLLT procedures also.

ADVANTAGES:

1. Relatively bloodless surgical and post-surgical course
2. The ability to coagulate, vaporize, or cut tissue
3. Sterilization of wound tissue
4. Minimal swelling and scarring
5. No requirement of sutures
6. Little mechanical trauma
7. Reduced surgical time
8. Decreased post-surgical pain
9. High patient acceptance[39]

DISADVANTAGES:

1. The high financial cost of a laser apparatus
2. Each laser has different characteristics because of their different wavelengths.
3. Possible damage to the underlying bone and dental pulp should also be considered. [39]

Ben-Bassat et al 1978 performed laser surgery for oral leukoplakia for the first time.He did LASER ablation of leukoplakia lesion of the tongue which marked the beginning of use of LASER use in oral cavity.[42]

Vedtofte E et al 1987 A study was done on 61 patients with oral premalignant lesion which were treated by surgical excision.The surgically created defects were closed by direct approximation of the wound edges in 25 patients,transposition by a local mucosal flap in 9, covered with a free mucosal graft in 3, and by a free split skin transplant in 24 patients.The patients have been followed for an average period of 3.9 years after the operation.A recurrence rate of 20% was found and three carcinomas developed in the follow up period. In conclusion, the experiences in using surgical excision for the treatment of premalignant lesions have been satisfying, and the importance of histological evaluation of the entire excised lesion has been documented.[3]

Roodenburg JLN etal 1991 treated total of 70 patients with 103 oral leukoplakias with carbon dioxide laser evaporation.This resulted in an excellent wound healing with virtually no scarring.The patients were followed up during a period of up to 12 years(mean 5.3 years), showing a cure rate of 90%.[43]

Thomson P. J et al 2002 did a study, in order to determine the efficacy of interventional CO_2 laser surgery in oral precancerous management,the records of 57 consecutive laser-treated patients presenting over a 4-year period,with histologically confirmed dysplastic lesions,were reviewed.Leukoplakias were the commonest clinical lesions (69%), with floor of the mouth was the most frequent anatomicalsite (42%).Laser surgery successfully excised 55 precancerous lesions, 11 of which exhibited more severe dysplasia or neoplasia compared with initial biopsy. Postoperative scarring and morbidity

were minimal.After surgery, patients were followed for between 1 and 44 months (mean 18 months).Out of these patients, 76% remained disease-free, whilst 24% developed new dysplastic lesions at distinct or multiple sites, often exhibiting increased dysplasia.Out of these patients experiencing recurrence, 7% developed oral squamous cell carcinoma, while a further 3.5% presented with other aerodigestive tract cancers. Neither initial lesion appearance nor histological diagnosis predicted clinical behavior.Interventional laser surgery is thus advised, in contrast to conservative management of oral precancerous lesion, to facilitate efficacious, low-morbidity treatment.[4]

Ishii J at al 2003 used different type of laser (CO_2, Nd- YAG and KTP) in the treatment for oral leukoplakia.The wound healing process after laser surgery was satisfactory and no significant complication were observed.The recurrence rate was 29.3% and malignant transformation was 1.2%.The review on laser surgery in treatment of oral leukoplakia reported the recurrence rate of oral leukoplakia 7.7 to 38.1%, while malignant transformation was 2.6 to 9%. The advantage of laser surgery for oral mucosal disease are hemostatic effects, minimal damage to adjacent tissue, excellent wound healing and limited contraction.Disadvantages of laser surgery was biopsy must be obtained preoperatively or at the time of treatment with laser vaporization and it takes longer time to re-epithelialize following excisional surgery.[1]

Farzane et al 2006 conducted a study during the period of 2005-2011 on 17 patients with oral leukoplakia and were treated with Nd-YAG laser were followed-up.The result showed 12 patients showed complete response, 2 responded partially and three did not respond.[44]

Nasiruddin et al. 2007 has published a case report on usage of CO_2 laser in the management of diffuse leukoplakia lesion over the dorsal surface of tongue using 10,600 nm at 2W power, patient has been followed for 6 months and noticed complete healing with no functional or neural deficit. Author also proposed that areas with less water content are resistant to vaporization with CO_2.So atmost care should be taken to remove even the deeper layers to encourage the regeneration of new epithelium and so prevent recurrence.[45]

Syed et al 2009 has evaluated CO_2 laser for excision of leukoplakia in comparison with traditional method in a total of 8 patients and noticed increased patient acceptance and operators comfort levels, with minimal postoperative complications.[46]

Vasavi et al 2009 in their study used CO_2 LASER for the management of leukoplakia lesion. 6 patiets in the age group 21-57years were included. Buccal mucosa was found to be the most affected site. They found good progression of healing. Mild pain and swelling was experienced by their patients which peaked between 72 hours and 1week.[47]

Kende Prajwalit at al 2011 in their study Diode laser was used for the removal of white patch on the tongue.The procedure was painless and no sutures were necessary.It was concluded that the use of diode laser is a promising aid in performing excisional biopsies of oral premalignant lesion,there was reduction of the operative time and postoperative discomfort. The patient was followed for next 6 month with no recurrence of the lesion.[5]

Raval Nilesh et al 2011 conducted a study on postoperative recovery, depth control and wound healing, 10 patients aged 40 to 65 years with oral symptomatic

leukoplakia and lichen planus was examined. Biopsy was taken before treatment as well as after complete healing. Patients were examined after 3 days, 1, 2 and 4 week after laser irradiation using visual analog score (VAS). Postoperative complication such as pain, bleeding, swelling, and functional disorder were graded 0 to 10. Out of the 10 patients, two patients (20%) complained of moderate pain during 3 days following laser irradiation, while rest of the patients (80%) complained of mild pain. The pain disappeared at the end of first week. Edema occurred in all cases. After 3 days edema in cheek was mild in one patient (10%), moderate in three patients (30%) and severe in four patients (40%).After 1st week, with improvement in pain and edema two patients (20%) showed return to normal function, four patients (40%) showed mild functional disturbance and there was no postoperative bleeding or scar formation and lased area was soft on palpation. During the third month follow up, out of 10 patients treated with diode laser (980nm), two patients complained of recurrence, while no recurrence was detected in rest of cases.[48]

Hristina et al 2012 implicated Nd-YAG diode laser at 10-15W power in a total of 17 patients diagnosed with leukoplakia. Complete healing was seen by the end of 4th week in most of the cases with no swelling noticed in any of the patient.[49]

Nihat Akbulut et al 2013 has evaluated the effects of the 810-nm diode laser in the treatment of 27 patients with various benign oral soft tissue lesions and noticed no complications in the surrounding soft tissue or hard tissue, with complete healing of white and vesiculobullous lesions noticed in duration of 6 weeks.[50]

SALIVARY ANTIOXIDANT EVALUATION

Salivary antioxidants are important to reduce the oxidative stress produced in the oral epithelium by the harmful products of tobacco. Superoxide Dismutase, Glutathione (peroxidase and reductase) are two antioxidants present in the saliva which are evaluated to correlate with the chance of malignant transformation.

Subha Gurudath et al 2012 evaluated serum SOD and GPx(peroxidase) in patients with Oral submucous fibrosis,oral leukoplakia and oral cancer. They have got statistically significant values which is much reduced when compared to the control group.[51]

Tajinder Kaur et al 2012 compared SOD and GSH levels in smokers and non-smokers.The mean value of SOD was significantly higher in smoking group than in non-smoking group but GSH-Px value was higher in non-smoker group than in smoking group.[52]

Rashmi Metgud and Saumya Bajaj 2014 in their study included 100 subjects comprising 30 apparently healthy controls, 30 patients with oral leukoplakia and 40 clinically and histologically diagnosed patients with OSCC. Saliva and blood samples were obtained and evaluated for MDA(Malondialdehyde) and GSH. The study revealed enhanced MDA levels in saliva and serum in oral leukoplakia and OSCC patients as compared to controls. On the other hand, significant decreases were seen in serum and salivary GSH levels in oral leukoplakia and OSCC patients as compared to controls. Augmentation of OS in blood and saliva is reflected by increase in MDA and decrease in

GSH levels, indicating that tumor processes cause an imbalance of oxidant-antioxidant status in cell structures.[53]

Hanspal Singh et al 2014 found significant changes in saliva of clinical staging and histological grading of oral squamous cell carcinoma (SCC) patients. Salivary SOD level between well to poorly differentiated SCC showed a progressive increase although it is not statistically significant.[54]

MATERIALS AND METHODS

SOURCE OF DATA:

Patients visiting the department of Oral Medicine and Radiology at the KSR institute of dental science & research, Tiruchengode, Namakkal dist, Tamil Nadu.

METHOD OF COLLECTION OF DATA:

The study protocol was analyzed and approved by the institutional ethical review board. Thirty systemically healthy patients were included in the study.The patients were having smoking habit and clinically and histopathologically proven leukoplakia.The patients were selected based on inclusion and exclusion criteria, those who were willing to participate in the study.The need and outcome of the treatment was explained to the patients and a written consent was obtained.

INCLUSION CRITERIA:

a. Systemically healthy subjects.

b. Clinically and histologically proven leukoplakia

c. Any sites in oral cavity except tongue

d. Any sized lesion

EXCLUSION CRITERIA:

1. Medically compromised patients

2. Pregnant and lactating women

3. Lesions on the tongue
4. Histologically carcinoma in situ and established carcinoma

STUDY DESIGN:

A total of thirty patients were enrolled in the study. All were male patients having clinically and histopathologically proven leukoplakia. The total of thirty patients were divided into two groups of 15 each.One group was subjected to antioxidant treatment and other group was subjected to diode LASER treatment. Before the treatment and after 3 months follow up salivary antoxidants like SOD and GSH values are evaluated for both groups.

CLINICAL EVALUATION OF THE PATIENTS:

Patients visiting KSR dental college is taken up for the study. Patients are subjected to routine clinical evaluation. In the routine examination, if any potentially malignant lesions are observed, they are subjected to detailed examination. Patients with oral leukoplakia are screened from those patients by clinical evaluation. A detailed proforma for history, clinical examination, histopathology and prognosis evaluation is used for recording those data. Initially the detailed history of the patient is taken including demographic data and habits. Clinical evaluation is the next step which includes the site, size, number and nature of leukoplakia. After that patients are subjected to incisional biopsy and histopathological examination. Routine blood investigations are done before incisional biopsy. If the patients are referred from a center outside where histopathological examination is already done, the reports are collected.

Thirty such patients are selected for the study after excluding many patients.The thirty patients are divided into two groups based on the treatment modality chosen.First group for conventional antioxidant treatment and the second group of patients are subjected to LASER ablation therapy. Informed and written consent are taken from all the patients.

TREATMENT WITH ANTIOXIDANTS

On the basis of lipid peroxidation effect and oxidative stress created by free radical, antioxidant treatment is advocated in leukoplakia patients.In our study we use Tab AtoZ(multi vitamin, folic acid and minerals) from Alkem Pharma to treat our patients. Patients were asked to quit the habit of smoking before commencement of therapy. An initial topical antifungal therapy was given to all the patients for one week to control candidal infection over added to leukoplakia lesions (Clotrimazole mouth paint-topical application for 7-10 days).The antioxidant therapy was advocated for three months using two tablets per day.Patient was followed up for three months.

TREATMENT WITH DIODE LASER

Diode LASER is taken as a second treatment option in our study.The selected patients are subjected to LASER ablation of the lesion.The patients are clinically evaluated in the third day,second week, first month and third month post operatively.Clinical evaluation of wound healing and post operative pain assesment using Visual Analogue Scale(VAS) is done.

POST TREATMENT CARE

All the patients treated with LASER are subjected to 3 days therapy of anti-inflammatory drug.The drug used was Ibuprofen and Paracetamol three times daily for 3 days.After the third day review if pain persisted the anti-inflammatory therapy is continued for two more days.Saline irrigation of the surgical site is done on third day of visit.If the wound healing is non-satisfactory after two weeks follow up,saline irrigation of the site is done in the visit.

SALIVARY ANTIOXIDANTS EVALUATION

Salivary antioxidants SOD and GSH are evaluated preoperatively and three months post operatively in LASER group.Same evaluation done before treatment and three month post treatment is done in Antioxidant group.Salivary antioxidant evaluation was done in Bio-Technology department of K.S.R College of Technology.

ARMAMENTARIUM

CLINICAL EXAMINATION

1. Mouth mirror
2. Probe
3. Explorer
4. Tweezer
5. Intraoral mirror
6. Measuring scale
7. Divider
8. Cheek retractor

9. Cotton pieces
10. Mask
11. Gloves

BIOPSY AND LASER ABLATION

1. All instruments for examination
2. Local anaesthetic solution
3. 2ml syringe and 21 gauge needle
4. Austin retractor
5. Bard Parker Blade No-15 and handle
6. Semiconductor diode laser
7. Safety glasses
8. Allis tissue holding forceps
9. Suture thread and needle
10. Needle holder
11. Saline cup
12. Gauze and cotton pieces

SALIVARY ANTIOXIDANT EVALUATION

1. Reagents
2. Spectrometer

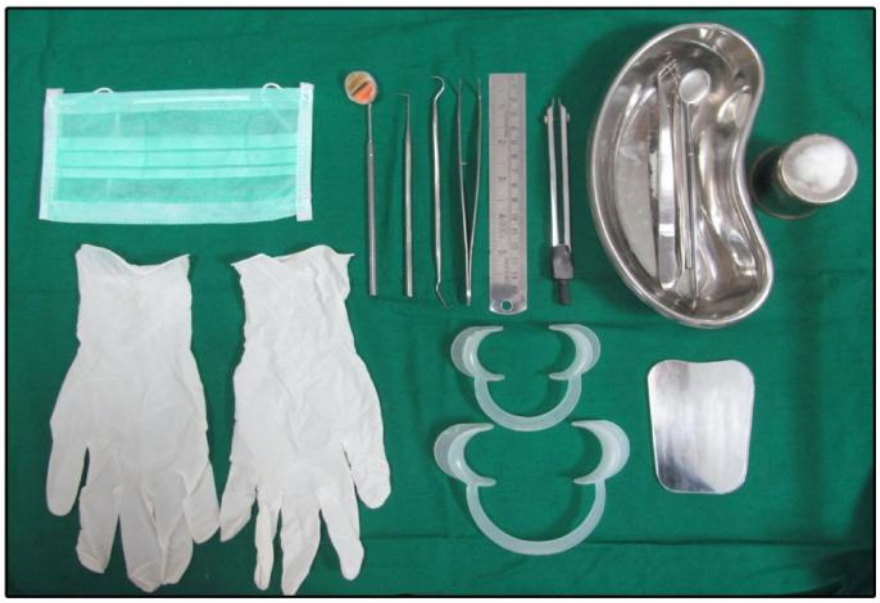


fig 1- Armamentarium for cilinical examination

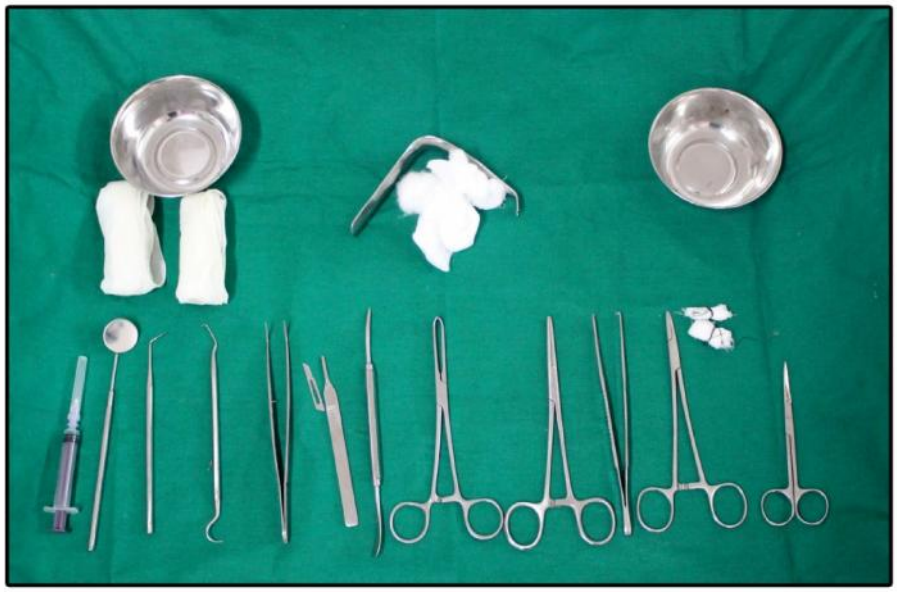


fig 2- Armamentarium for biopsy

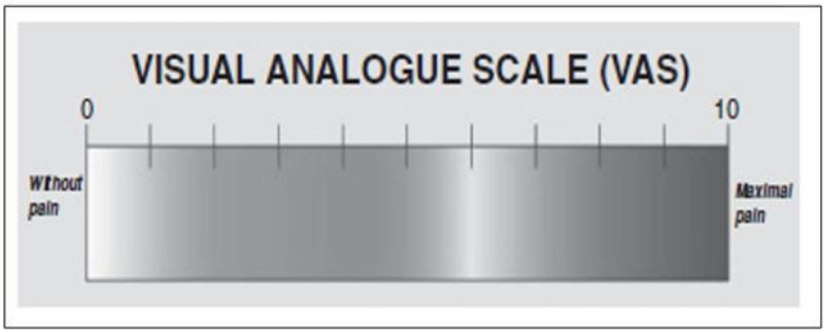


fig 3- Visual Analogue Scale

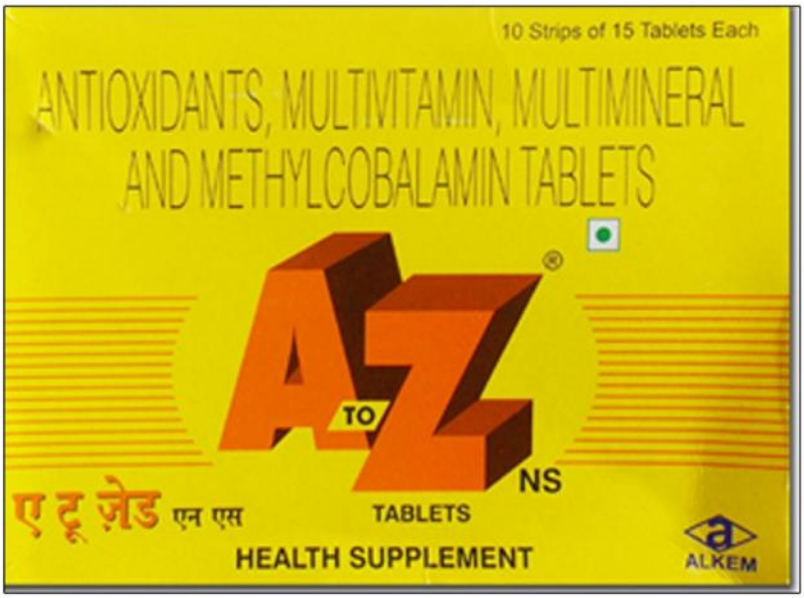


fig 4 -Antioxidant tablet AtoZ

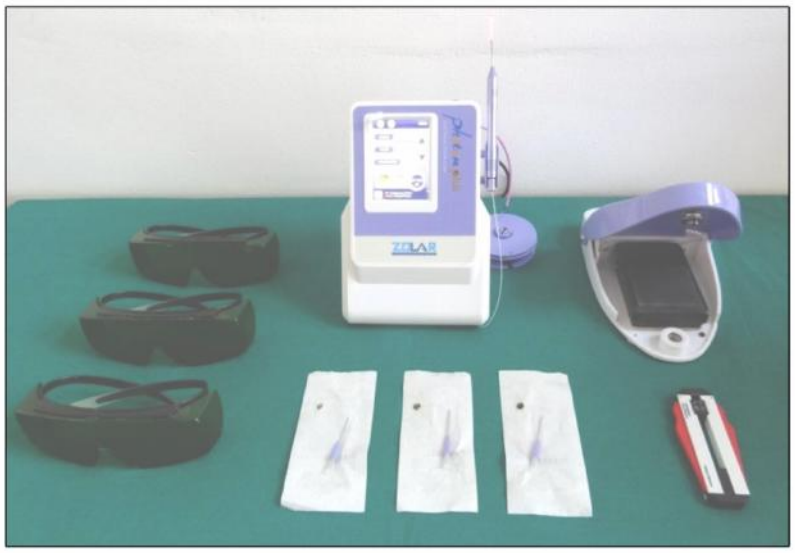


fig 5-Armamentarium for laser technique

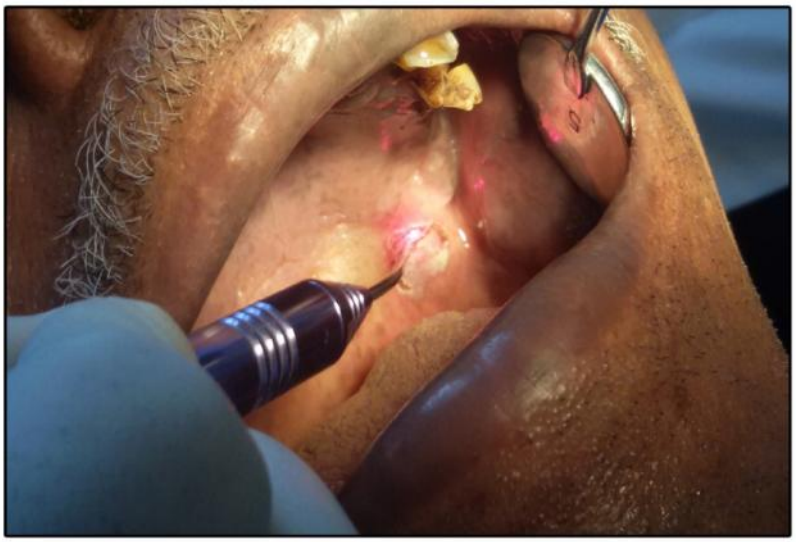


fig 6 -Laser ablation

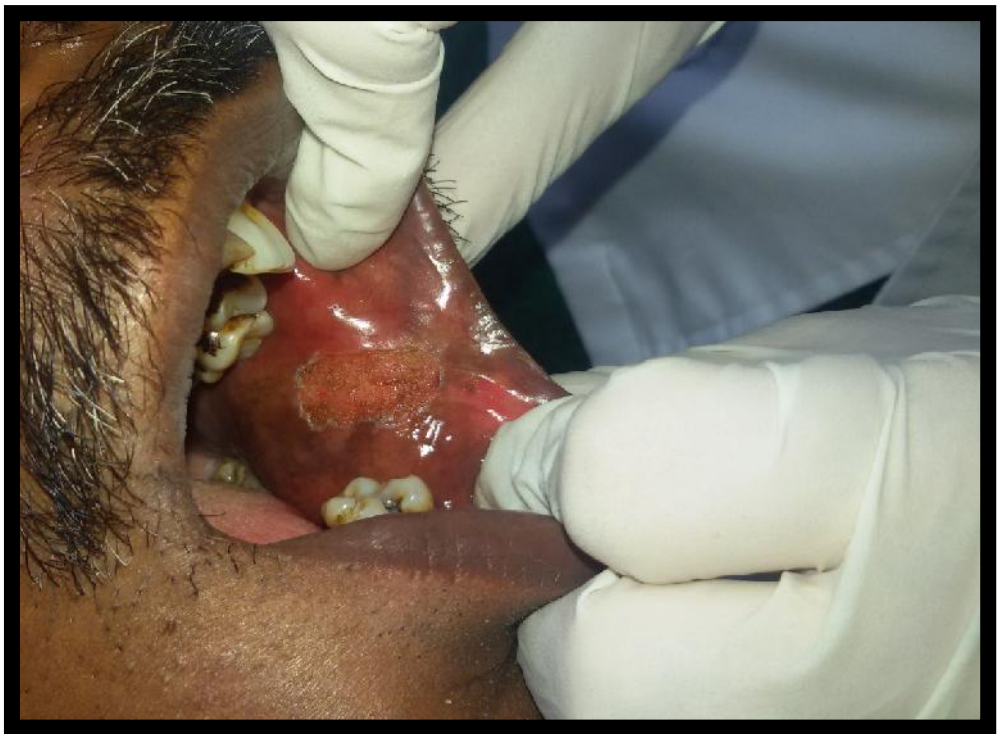


Figure 7- Immediate post-operative

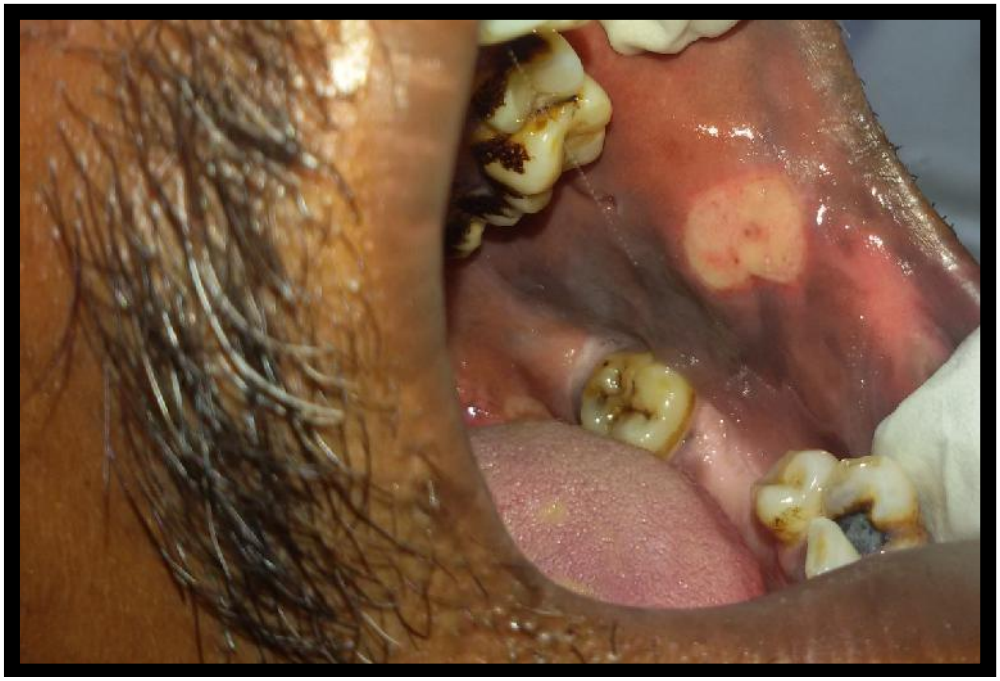


Figure 8- post-operative- 3days

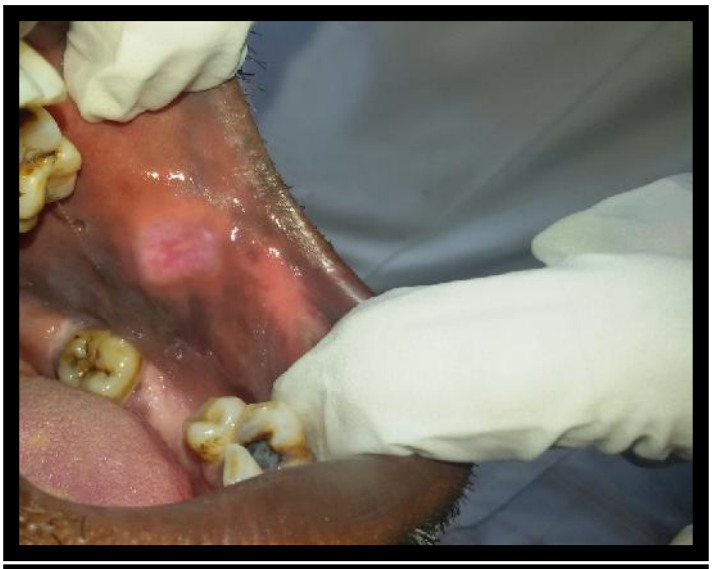


Figure 9- post-operative- 14days

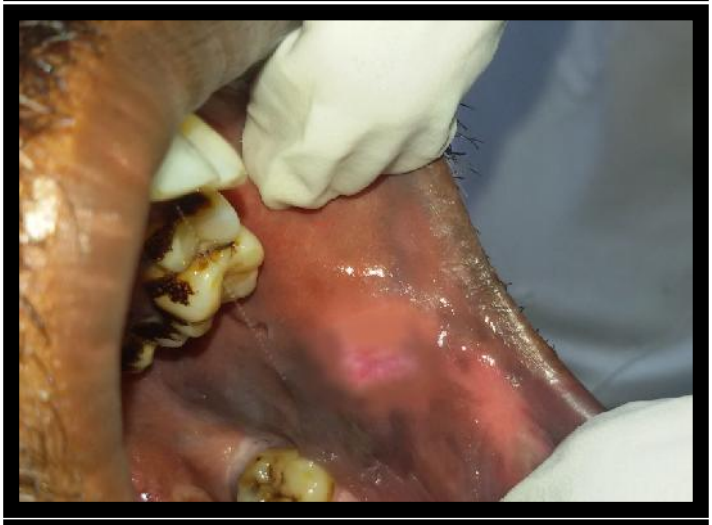


Figure 10- post-operative- 1month

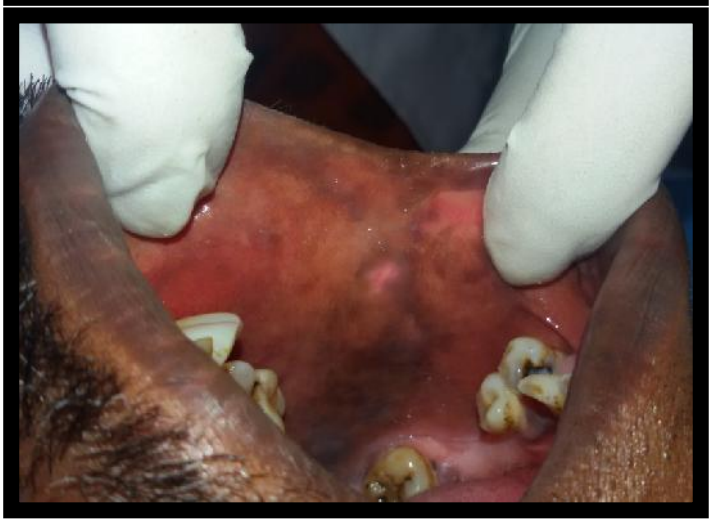


Figure 11- post-operative- 3month

STASTISTICAL ANALYSIS

The data obtained from the study was entered in Microsoft Excel and was given for statistical analysis.The data was analyzed using SPSS software. Student's t test was used to analyze the significance of the values of salivary antioxidants.

PAIRED t TEST:

Paired t test is applied when there is a pair of data from single element in an observation. Data are collected before and after the intervention, so that the same group acts as both case and control. Then the mean of both the groups are compared to get the t value.

RESULTS

AGE GROUP OF PATIENTS

Age	Frequency	Percentage
40-60	10	66.7
>60	5	33.3
Total	15	100

Table 1.1 Distribution of age among patients treated with antioxidants

Age	Frequency	Percentage
40-60	7	46.7
>60	8	53.3
Total	15	100

Table 1.2 Distribution of age among patients treated with diode laser

Table 1.1 and 1,2 give the inference that all the patients participated in the study were above 40 years of age and in that 43.3% were above 60 years of age.

GENDER

Gender	Frequency	Percentage
Male	15	100

Table 2.1 Distribution of gender among patients treated with antioxidants

Gender	Frequency	Percentage
Male	15	100

Table 2.2 Distribution of gender among patients treated with diode laser

Table 2.1 and 2.2 shows that all patients participated in the study were males in both the groups

FORM OF TOBACCO

Form	Frequency	Percentage
Cigrette	4	26.7
Beedi	11	73.3
Total	15	100

Table 3.1 Usage of tobacco among patients treated with antioxidants

Form	Frequency	Percentage
Cigrette	5	33.3
Beedi	10	66.7
Total	15	100

Table 3.2 Usage of tobacco among patients treated with diode laser

Patients were classified as cigarette smokers and beedi smokers in this study.70% of total subjects were beedi smokers.In antioxidant group it is 73.3% and in LASER group it is 66.7%.

NUMBER OF CONSUMPTIONS PER DAY

Number	Frequency	Percentage
<5	3	20
5-10	6	40
>10	6	40
Total	15	100

Table 4.1 No of consumptions of tobacco (per day) among patients treated with antioxidants

Number	Frequency	Percentage
<5	3	20
5-10	6	40
>10	6	40
Total	15	100

Table 4.2 No of consumptions of tobacco (per day) among patients treated with diode laser

The categories were 1-less than 5 per day, 2- 5 to 10 per day, 3- more than 10 per day. 40% of patients were in second and third category. The rest (20%) were in the first.

YEARS OF CONSUMPTION

Number of years	Frequency	Percentage
>10	15	100

Table 5.1 No of years of consumption of tobacco among patients treated with antioxidants

Number of years	Frequency	Percentage
>10	15	100

Table 5.2 No of years of consumption of tobacco among patients treated with diode laser

1- less than 5 years, 2- 5 to 10 years and 3- more than 10 years were the grouping for this parameter. all the patients were in third group who were smoking more than 10 cigarettes/beedi per day.

NUMBER OF LESIONS

No. of lesions	Frequency	Percentage
1	8	53.3
2	7	46.7
Total	15	100

Table 6.1 No of Lesions among patients treated with antioxidants

No. of lesions	Frequency	Percentage
1	14	93.3
2	1	6.7
Total	15	10

Table 6.2 No of Lesions among patients treated with diode laser

Table 6.1 an 6.2 give an inference that 73.3% of patients in the study were having only single lesions and 26.6% were having multiple lesions.

SITE

Site	Frequency	Percentage
Buccal mucosa	15	100

Table 7.1 Site of Lesion among patients treated with antioxidants

Site	Frequency	Percentage
Buccal mucosa	13	86.7
Labial mucosa	1	6.7
Palate	1	6.7
Total	15	100

Table 7.2 Site of Lesion among patients treated with diode laser

The distribuition of site of the lesion are given i Table 7.1 and 7.2. Buccal mucosa-1, Labial mucosa-2 ,Palate-3 and Other sites-4 were the grouping. the results shows that 93.3% of patients were having lesions in the buccal mucosa(28/30).

SIZE OF LESION

Size	Frequency	Percentage
<2	2	13.3
2-4	10	66.7
>4	3	20
Total	15	100

Table 8.1 Size of Lesion among patients treated with antioxidants

Size	Frequency	Percentage
<2	2	13.3
2-4	12	80.0
>4	1	6.7
Total	15	100

Table 8.2 Size of Lesion among patients treated with diode laser

Lesion size in the oral cavity were grouped into three 1-less than 2cm, 2- 2 to 4 cm and 3-more than 4 cm.Table 8.1 and 8.2 shows that 13.3% were in group 1, 73.3% in group2 and the rest 13.3% in group 3.

NATURE OF LESION

Nature	Frequency	Percentage
Homogeneous	15	100

Table 9.1 Nature of Lesion among patients treated with antioxidants

Nature	Frequency	Percentage
Homogeneous	14	93.3
Non- Homogeneous	1	6.7
Total	15	100

Table 9.2 Nature of Lesion among patients treated with diode laser

Table 9.1 and 9.2 shows that 96.7% of patients are in group 1 which included lesion of homogenous type and rest9one patient) was having non homogenous type of leukoplakia.

DYSPLASIA

Nature of dysplasia	Frequency	Percentage
Mild	10	66.7
Moderate	5	33.3
Total	15	100

Table 10.1 Nature of Dysplasia among patients treated with antioxidants

Nature of dysplasia	Frequency	Percentage
Mild	11	73.3
Moderate	2	13.3
Severe	2	13.3
Total	15	100

Table 10.2 Nature of Dysplasia among patients treated with diode laser

Table 10.1 shoes the distribuition of patients according to epithelial dysplasia in antoxidant group and Table 10.2 shows distribuition in LASER group.Out of 30 patients 21 patients (70%) were having mild dysplasia, 7 patients(23.3%) in moderated dysplasia and 2 patients (6.6%) in severe dysplasia group

CONTINUATION OF HABIT AFTER COMMENCEMENT OF TREATMENT

Habit	Frequency	Percentage
Reduced	3	20
Quit	12	80
Total	15	100

Table 11.1 Continuation of habits among patients treated with antioxidants

Habit	Frequency	Percentage
Reduced	4	26.7
Quit	11	73.3
Total	15	100

Table 11.2 Continuation of habits among patients treated with diode laser

23/30 patients (76.6%) quit smoking after the commencement of smoking.The rest of the patients did not quit smoking but has reduced the frequency and number.

REGRESSION OF LESION SIZE

Regression	Frequency	Percentage
No	15	100

Table 12.1 Regression in size of lesion among patients treated with antioxidants

Regression	Frequency	Percentage
Yes	15	100

Table 12.2 Regression in size of lesion among patients treated with diode laser

Tables 12.1 shows that all the patients were in the group of no reduction in lesion size in antioxidant group and Table 12.2 shows in LASER group the lesion has completely disappeared.

FORMATION OF NEW LESION

Formation	Frequency	Percentage
Yes	15	100

Table 13.1 Formation of new lesion among patients treated with antioxidants

Formation	Frequency	Percentage
No	15	100

Table 13.2 Formation of new lesion among patients treated with diode laser

MALIGNANT TRANSFORMATION

Malignant transformation	Frequency	Percentage
Yes	15	100

Table 14.1 Malignant transformation among patients treated with antioxidants

Malignant transformation	Frequency	Percentage
No	15	100

Table 14.2 Malignant transformation among patients treated with diode laser

Table 13 and 14 represents formation of new lesion and malignant transformation in both groups. The results suggest that in both groups there are no new lesions formed and no malignant transformation observed clinically.

CHANGE IN NATURE

Nature	Frequency	Percentage
Erythematous	1	6.7
No change	14	93.3
total	15	100

Table 15.1 Change in nature of lesion among patients treated with antioxidants

Nature	Frequency	Percentage
No change	15	100

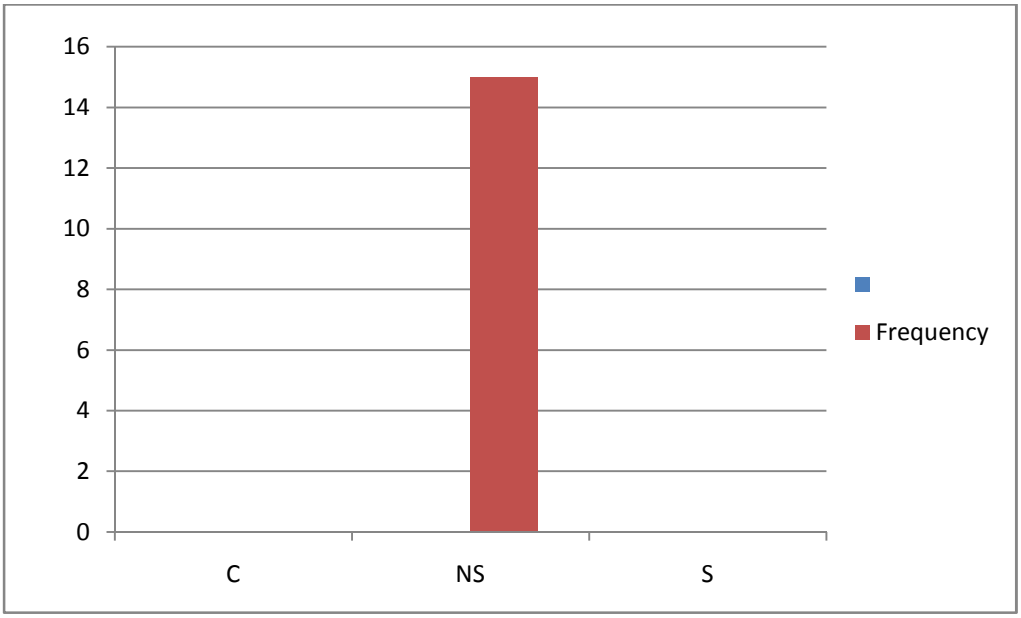
Table 15.2 Change in nature of lesion among patients treated with diode laser

Change in nature of lesion was examined in the study and Table 15.1 and 15.2 represents the groups. only one patient in antioxidant group shows change in nature of lesion

WOUND HEALING AFTER LASER SURGERY

Third day	Frequency	Percentage
Non satisfactory	15	100

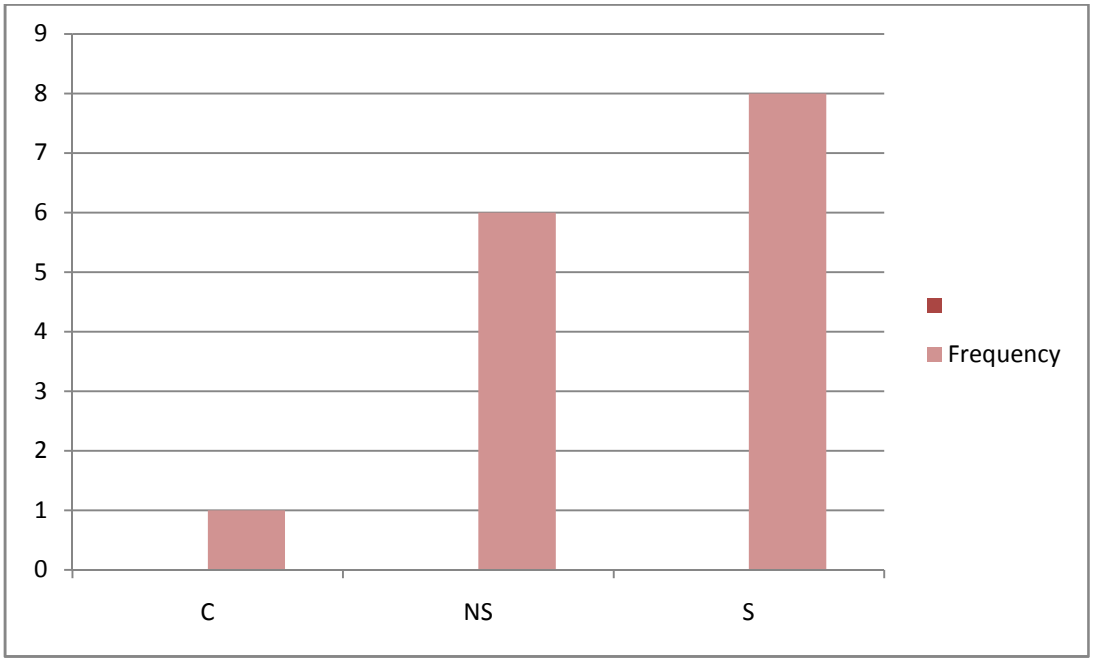
Table 16.11 Wound Healing (3 days) among patients treated with diode laser



Graph 1.1 Wound Healing (3 days) among patients treated with diode laser

Second week	Frequency	Percentage
Complete	1	6.7
Non satisfactory	6	40
Satisfactory	8	53.3
Total	15	100

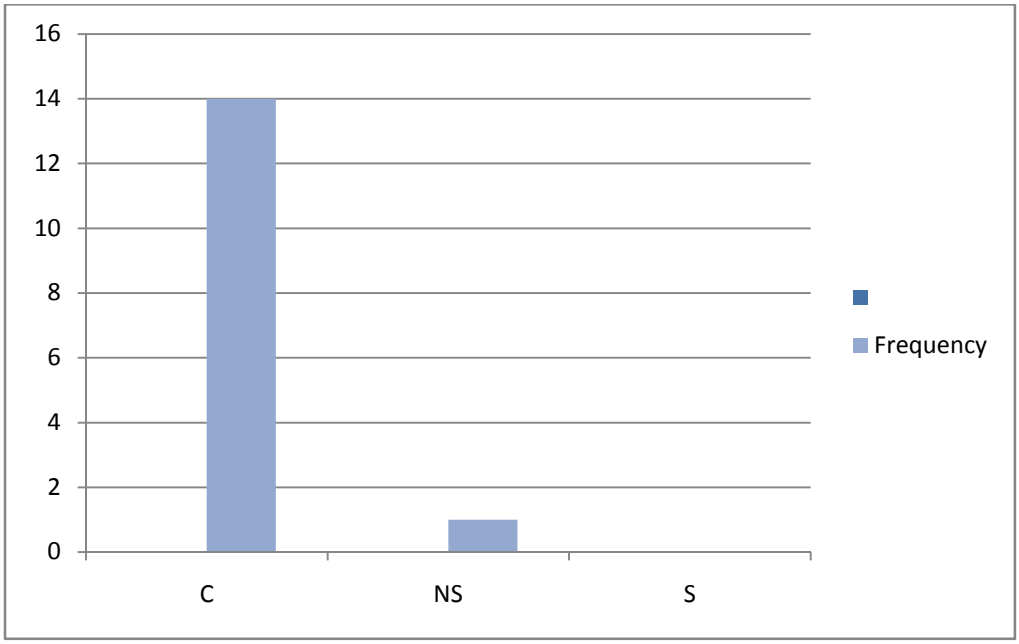
Table 16.12 Wound Healing (2 weeks) among patients treated with diode laser



Graph 1.2 Wound Healing (2 weeks) among patients treated with diode laser

First month	Frequency	Percentage
Complete	14	93.3
Non satisfactory	1	6.7
Total	15	100

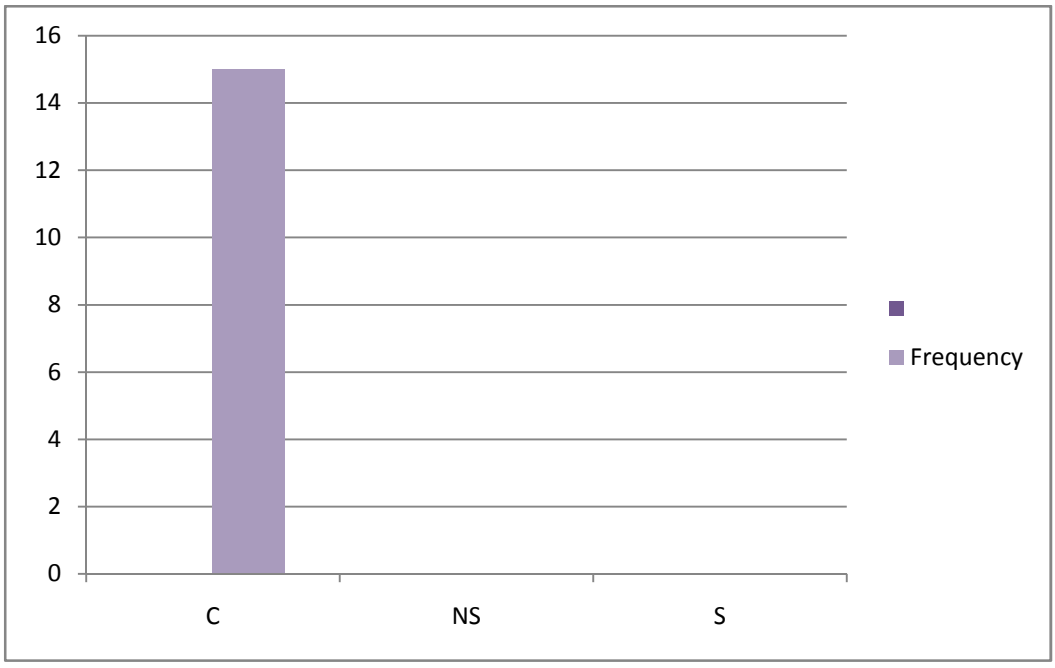
Table 16.13 Wound Healing (1 month) among patients treated with diode laser



Graph 1.3 Wound Healing (1 month) among patients treated with diode laser

Third month	Frequency	Percentage
Complete	15	100

Table 16.14 Wound Healing (3 months) among patients treated with diode laser



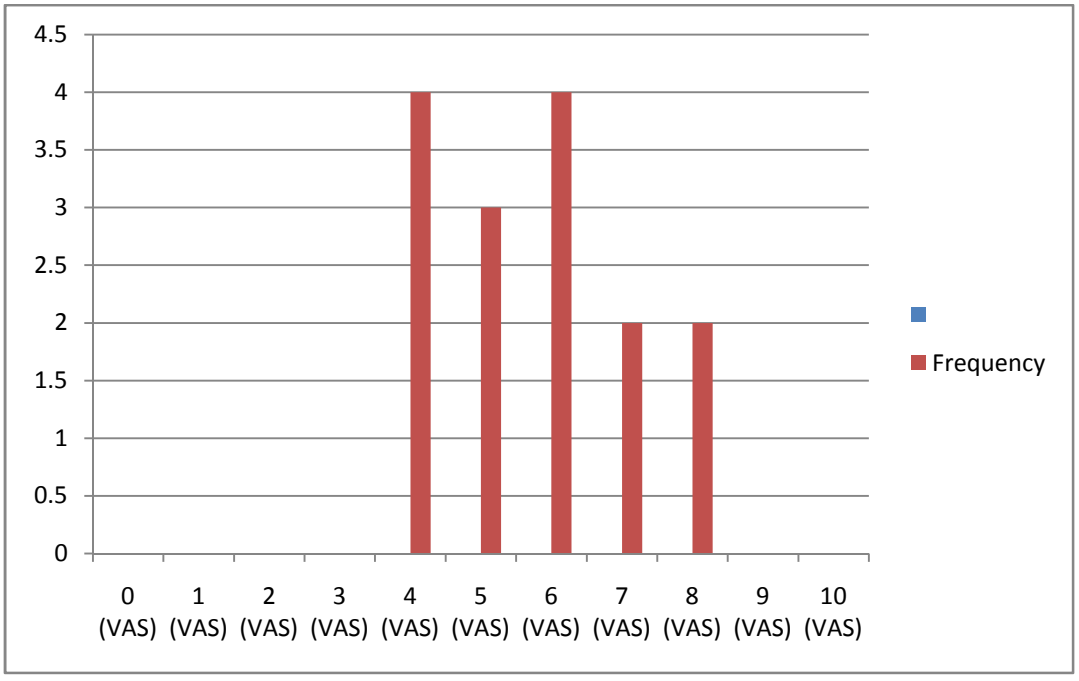
Graph 1.4 Wound Healing (3 months) among patients treated with diode laser

Table 16 and Graph 1 shows clinical evaluation of wound healing in patients in all the follow up period from third day to third month. From total non-satisfactory healing it progressed to almost complete healing in one month follow up except in one patient.In the third month follow up complete wound healing was noticed in all the patients

PAIN IN VAS

Pain in VAS	Frequency	Percentage
4	4	26.7
5	3	20.0
6	4	26.7
7	2	13.3
8	2	13.3
Total	15	100

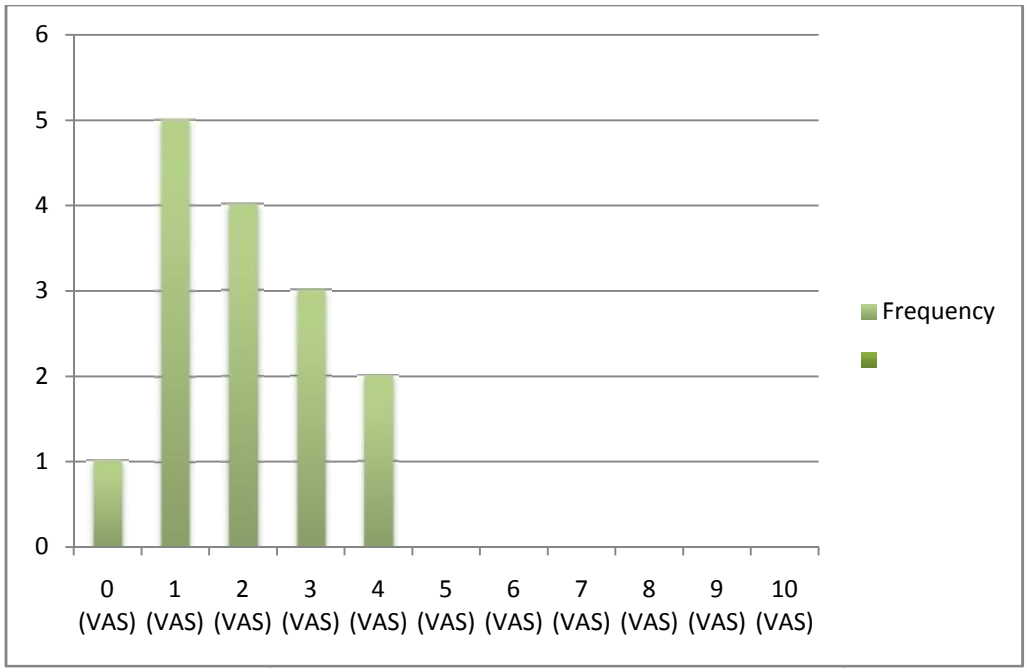
Table 17.11 Pain in VAS (3 days) among patients treated with diode laser



Graph 2.1 Pain in VAS (3 days) among patients treated with diode laser

Pain in VAS	Frequency	Percentage
0	1	6.7
1	5	33.3
2	4	26.7
3	3	20.0
4	2	13.3
Total	15	100

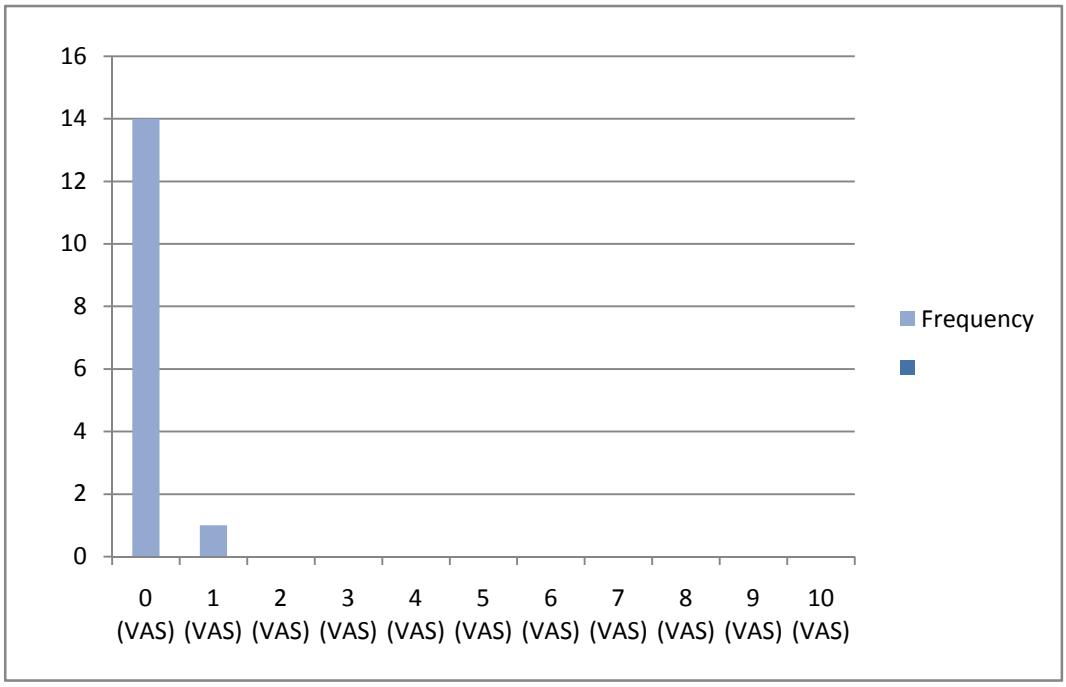
Table 17.12 Pain in VAS (2 weeks) among patients treated with diode laser



Grapg 2.2 Pain in VAS (2 weeks) among patients treated with diode laser

Pain in VAS	Frequency	Percentage
0	14	93.3
1	1	6.7
Total	15	100

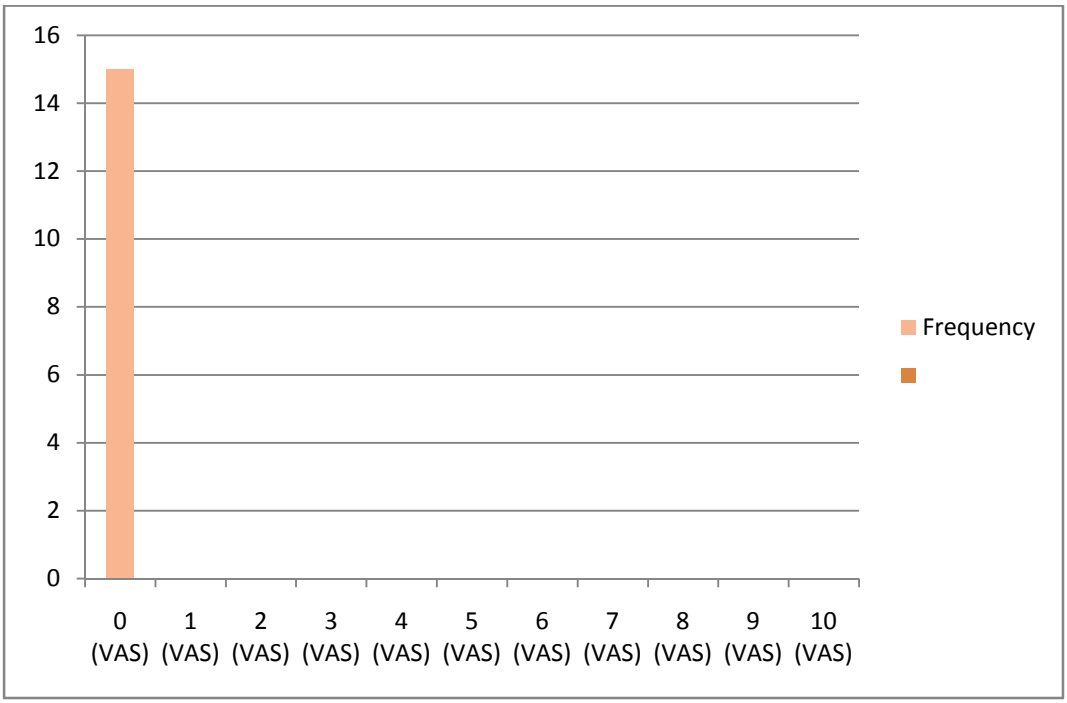
Table 17.13 Pain in VAS (1 month) among patients treated with diode laser



Graph 2.3 Pain in VAS (1 month) among patients treated with diode laser

Pain in VAS	Frequency	Percentage
0	15	100

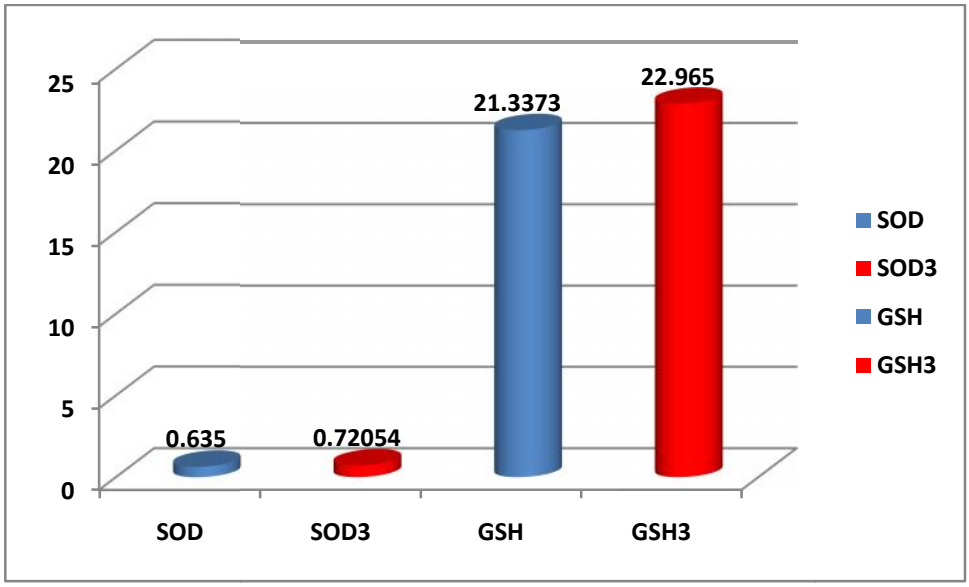
Table 17.14 Pain in VAS (3 months) among patients treated with diode laser



Graph 2.4 Pain in VAS (3 months) among patients treated with diode laser

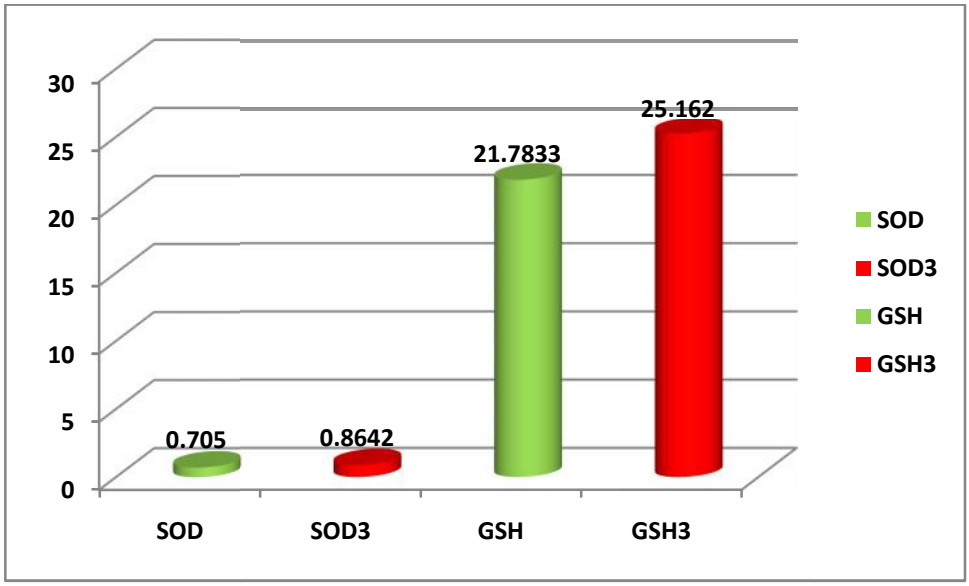
Table 17 and Graph 2 shows the VAS pain records of the patients.Gradual decrease in pain can be noted from the results.In 2 weeks the pain totally disappeared for almost all the patients

ANTIOXIDANT: SALIVARY ANTOXID LEVELS



Graph 3: mean salivary SOD and GSH levels before treatment and three months after treatment.

LASER: SALIVARY ANTOXID LEVELS



Graph 4: mean salivary SOD and GSH levels before treatment and three months after treatment.

Student T-test results for salivaru antioxidant evaluation

Antioxidant	t	df	P-value
Pair 1 SOD - SOD3	**-5.021**	**14**	**.030**
Pair 2 GSH - GSH3	**-6.990**	**14**	**.042**

Table 18.1 P-value evaluation in group of patients treated with antioxidant

LASER	t	df	P-value
Pair 1 SOD - SOD3	**-7.420**	**14**	**.020**
Pair 2 GSH - GSH3	**-7.194**	**14**	**.012**

Table 18.2 P-value evaluation in group of patients treated with Diode LASER

Graph 3 and 4 gives an inference about the marked increase of mean valued of both salivary antioxidant after three months follow up. Student-T test was performed using mean value and standard deviation values. The results of that test is depicted in Table 18.1 and 18.2 which shows the p-value obtained. Any p-value.<.05 is considered as statistically significant. all the values are found to be significant in this study statistically.

DISCUSSION

Leukoplakia is considered as one of the most common potentially malignant lesion presenting in the oral cavity which has a fair rate of malignant transformation. Various treatment modalities have been suggested for oral leukoplakia. Antioxidants in various forms have been tried in treating oral leukoplakia since many years. LASER ablation has gained importance presently because of its superior prognostic effects compared to antioxidants and conventional surgical management.

Salivary antioxidants are important in preventing cellular damage by oxidative stress. Superoxide Dismutase and Glutathione are responsible for reducing the harmful effects of reactive oxygen species. These antioxidants can be considered as a salivary biomarker of progression of cellular damage.

All the patients who participated in our study were males. Previous studies in Indian population, support this analysis in our study; **Sankaranarayanan et al (1997),Silverman et al(1976) and Van der Hem et al(2005)** but it is in contrast to report given by **Garewal et al 1999** which showed female predominance. This shows,the male predominance of the lesion. The increased prevalence in males can be attributed to the difference between smoking habit in males and females. All the patients in our study were smokers. In south Indian population using tobacco in the form of smoke is notably more in males than in females.[21,23,55,56]

The patients participated in the study were above 40 years of age in both the groups.In this 43.3% of patients were above sixty years of age.The age group in our study

was found to be similar to that of **Syed et al(2009)**,**Vivek et al(2008)** and **Vasavi et al(2009)** in the study group treated using Diode LASER.In patients treated with antoxidants, studies in similar age group was performed by **Sankaranarayanan et al 1997, Lippman et al 1993.**Two inferences can be made from this data-one is the long time period between the commencement of smoking and formation of the leukoplakia patch in oral cavity, the second inference is the fact that the leukoplakia lesion is almost always unnoticed by the patients.Most of the detection is during a routine dental procedure or a dental visit for some other complaint.This has to be taken into serious consideration,since leukoplakia is a potentially malignant condition and there is a fair rate of malignant transformation.[21,46,47,31]

TOBACCO CONSUMPTION

All the patients in our study were using tobacco in the form of smoke; either cigarette or beedi. 70% of the patients were using beedi. More advanced lesions regarding size, homogenecity and also histopathological dysplastic grade can be related to extensive beedi use.A cross sectional study of a larger sample size is required to prove the harmful effects of beedi compared to cigarettes..The cigarette smokers in our study were using cigarettes with filters and that can be also a reason for this difference.

Regarding the number of consumptions per day, 80% of patients were consuming more than 5 cigarette/beedi per day.In that 50% of patients were consuming more than 10 cigarette/beedi per day.This value signifies the direct co-relation between amount of tobacco use and the formation of leukoplakia lesion. Regarding the number of consumption in all the groups, beedi smokers predominated.

Number of years of consumption is another parameter which was queried in our study.All the participants fell into the group of more than 10 years of consumption with a mean of 23 years of consumption..The chance of getting a higher degree of dysplasia histopathologically and increased risk of getting a field effect should be considered seriously in this context.

CLINICAL EXAMINATION OF THE LESION

27% of patients (8 out of 30) were having multiple lesions and the rest were having only a single lesion.Out of those patients, six were beedi smokers(75%).Most of the multiple lesions found were with bilateral presentation in the buccal mucosa.Similar inference was made by **J.Ishii et al 2003** in their study of 116 patients(77.6%-single lesion).It can be inferred from this result that beedi smokers are at a higher risk of getting multiple lesions.[1]

Buccal mucosal lesion predominated in our study among patients.93% of patients were having buccal mucosal lesions. **Pindborg et al**(85.5%), **silverman et al 1976**(77%), **Sebnam et al 2006**(61%) have got similar results in the studies on Indian popuation.In the studies conducted in United States and Europe,distribuition of lesion in buccal mucosa is found to be less compared to Indian population (**Roodenburg et al 1991 and Silverman et al1968**). Two patients were having extra buccal mucosal lesions-one in the palate and another in the labial mucosa. The patient with palatal lesion had a co-existing buccal mucosal lesion and he was a beedi smoker.This data relates the findings of previous studies that the most common site for oral leukoplakia is buccal mucosa. (ref 32 26 27). One more fact of increased number in buccal mucosa in our study was that we have excluded leukoplakia lesions affecting tongue and the floor of the mouth.[43,57,58,59]

73.3% of patients fell into the group of lesion of sizes varying from 2cm-4cm.13.3% patients were under the group of more than 4cm and 13.3% were under the group of less than 2 cm.This result is similar to one of the largest studies by **P.S Van der Hem et al 2005**(288 leukoplakia lesions).The size of leukoplakia lesions can be variable. Strict co-relation between size and other parameters are not established yet. The patients with lesion size more than 4 cm fell equally into groups of beedi and cigarette smokers.[56]

Most common form of oral leukoplakia is homogenous form.97% of patients who participated in our study were having homogenous form of leukoplakia. Only one patient had a non-homogenous form,which presented as a mixed red and white lesion in a beedi smoker. The histopathological analysis proved it to be having severe dysplasia. Previously, the studies by **P.S Van der Hem et al 2005(69% homogenous), Syed et al 2009 (75% homogenous)** showed similar results. This shows that in leukoplakia lesions with erythematous component the grade of dysplasia tend to be higher.[56,42]

HISTOPATHOLOGY

Patients who were clinically diagnosed as having leukoplakia were selected for the study and were subjected to histopathological examination. Histopathologically, they were graded according to the degree of dysplasia as mild, moderate and severe.70% patient with mild dysplasia, 23% with moderate dysplasia and 7% with severe dysplasia were present in our study. This result is similar to that of **P.S Van der Hem et al 2005** in which more than 50% of the patients with dysplasia fell into category of mild dysplasia**. Praveen Birur et al 2014** in their study tried to relate nature of leukoplakia and degree of dysplasia and they concluded that grades of dysplasia does not depend on clinical

nature of the lesion. This report is in accordance with the results obtained in our study.[56,60]

All the patients with severe dysplasia were beedi smokers and one of them had more than 4 cm sized lesion. In patients with moderate dysplasia 75% were beedi smokers and 37.5% patients were having lesion size more than 4 cm.

PROGNOSIS EVALUATION

The three months follow up showed no regression in size of the lesion noticed in patients treated with antioxidants. Studies by **Lipmann et al 2006, Liede et al 1998** reported similar finding. Regression in size of the lesion in patients treated with antioxidants were reported by many other studies (**Piatelli et al 1999** (10%), **Dimery et al** 1997 (71%), **Scardina et al** (85%),**Benner et al1993** (20%)).[31,32,33,22,20]

There are chances for the formation of new lesion in patients who are treated for leukoplakia. Continuation of the habit and multiple field of cancerization can be related to the new lesion formation. Patients presenting with multiple lesions simultaneously, are examples for multiple field effect. According to the results obtained, new lesion formation could not be identified in any of our patients in three months follow up. This included patients who did not quit smoking after commencement of treatment also.

In our study we have included recurrent lesions in the same site and also a second primary lesion as a newly formed lesion. Our results are similar to the results obtained in their studies using Antioxidants by **Garewell et al 1990, S.Toma et al 1992,Tradati et al 1994 and Dimery et al 1997.**In contrast to this finding various recurrence rate of the

lesion were mentioned by **Stich et al 1988** (21%), **Sankaranarayanan et al 1997** (67**%), Garewell et al 1999** (18%).[13,15,17,20,21]

In the study group in which Diode LASER was used,complete absence of recurrence is not reported in any of the major studies, **J.W.Frame et al 1984** reported .05% recurrence is the closest co-relation to our study. **Silverman et al 1988** (10.8%), **Roodenburg et al 1991** (10%) **Schoelch et al 1999** (38.1%), **Ishii et al 2002** (29.3%) are the other similar studies showing recurrence rate.[1,43,56,61]

Leukoplakia is a potentially malignant lesion and there is a varying malignant transformation rate from 0.13% to 15.7% (Tradati et al 1997; Oral Oncol).Clinical evaluation of malignant transformation was evaluated in our study. Ulcerative or an Ulcero-proliferative growth with or without regional lymph node involvement was the criteria.None of the patients in our study showed malignant transformation clinically. Only one patient in the antioxidant group showed mild erythematous change in the previously found homogenous leukoplakia.

In antioxidant group this result is in accordance with the results given **by Toma et al 1992, Stich et al 1988,Tradati et al 1994 and Sankaranarayanan et al 1997** but in contrast with the reports given by **Garewell et al 1990** (8%), **Garewell et al 1999** (38%), **Lipmann et al 2006** (23%).In the previous studies using Diode LASER by **Roodenburg et al 1991, Vivek et al 2008 and Vasavi et al 2009** 0% malignant transformation rate was reported,which is similar to our study and in contrast **Silverman et al 1988** reported 2.6% and **Ishii.J et al 2003** reported 1.2% of malignant transformation rate.[1,13,19,21,23,46,47,57]

We evaluated the levels of salivary antioxidants SOD and GSH in the pre-treatment period and post follow up period of three months. The mean values given in the results Graph 3 and 4 suggest that there is marked increase in the value of both antioxidants after treatment of leukoplakia compared to pre-treatment stage. The p-values obtained by T-test suggest that the difference is significant statistically. When there is a marked lesion with continuing smoking habit the level of oxidative stress is very high and need of more salivary antioxidants suggest the increased value initially.

Later, after treatment, the existed lesion was ablated off using Diode LASER in one group and an additional support of antioxidants was given in the other group as therapy. Counseling was also given to the patients regarding the harmful effects of smoking and the need of quitting the habit. That has helped in reducing the smoking habit in our patients. Both quitting the habit and the therapy given would have directly helped in reducing oxidative stress and there by lipid peroxidation which would have lead to an increased salivary antioxidant level post-operatively after three months. The results of this analysis is in accordance with that of **Tajinder et al 2012,Hanspal Singh et al 2014.**[53,54]

SUMMARY AND CONCLUSION

We started our study with an aim to evaluate the efficacy of Diode LASER in treating patients with oral leukoplakia. We selected thirty patients who were clinically and histopathologically diagnosed as leukoplakia. The patients were grouped into two: one group of 15 patients who were managed using conventional antioxidants and another group of 15 treated using Diode LASER. Salivary antioxidants were evaluated in the pre-treated period and also after three months follow up.

The results were analyzed and we could find that there was complete resolution of lesion with minimum patient discomfort in the patients treated with Diode LASER. There was statistically significant difference in the salivary antioxidants levels between pre-treatment period and after three months follow up period. This difference was noted in patients treated with antioxidants and Diode LASER.

CONCLUSION

Diode LASER is superior to antioxidant therapy in the treatment of oral leukoplakia regarding clinical wound healing and resolution of the lesion. Salivary antioxidants, SOD and GSH can be considered as a valuable marker for knowing the treatment outcome and prognosis. We conclude here by emphasizing the need of further research in the field of oral potentially malignant lesions and salivary biomarkers.

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Annexure – I (PROFORMA)

LEUKOPLAKIA TREATMENT WITH ANTI-OXIDANTS

I Sl.No.:_____ OP No:_____ Age : _____ Sex : M / F

Name : _____

II HABITS

1. Form of Tobacco : Cigarette / Beedi

2. No. of Consumptions per day : _____

3. No. of years of consumption : _____

III CLINICAL DESCRIPTION OF LEUKOPLAKIA

1. No. of Lesions : _____

2. Site of Lesion : Buccal Mucosa / Labial Mucosa / Palate / Other sites

3. Size of the Lesion : < 2cm / 2-4cm / >4cm

4. Nature of the Lesion : Homogeneous / Non-Homogeneous

IV HISTOPATHOLOGY

Dysplasia - Mild / Moderate / Severe

V SALIVARY ANTIOXIDANT LEVEL : SOD : _____

GP : _____

VI CONTINUATION OF HABITS AFTER COMMENCEMENT OF TREATMENT

1. Continues Smoking : Yes / No

2. If yes : Same No as before / Reduced

VII PROGNOSIS OF TREATMENT

1. Regression in size of Lesion : Yes / No

2. Formation of New Lesion : Yes / No

3. Malignant Transformation : Yes / No

4. Change in the Nature of Lesion : Yes / No

   If yes, Erythematous / Proliferative

5. Adverse effects of Drug, if any : _____

6. Salivary Antioxidant level after 3 months : SOD : _____

   GP : _____

Signature of Trainee : _____ Signature of Guide : _____

Annexure - II (PROFORMA)

LEUKOPLAKIA TREATMENT WITH DIODE LASER

I Sl.No.:_____ OP No:_____ Age : _____ Sex : M / F

Name : _____

II HABITS

1. Form of Tobacco : Cigarette / Beedi
2. No. of Consumptions per day : _____
3. No. of years of consumption : _____

III CLINICAL DESCRIPTION OF LEUKOPLAKIA

1. No. of Lesions : _____
2. Site of Lesion : Buccal Mucosa / Labial Mucosa / Palate / Other sites
3. Size of the Lesion : < 2cm / 2-4cm / >4cm
4. Nature of the Lesion : Homogeneous / Non-Homogeneous

IV HISTOPATHOLOGY

Dysplasia - Mild / Moderate / Severe

V SALIVARY ANTIOXIDANT LEVEL : SOD : _____

GP : _____

VI CONTINUATION OF HABITS AFTER COMMENCEMENT OF TREATMENT

1. Continues Smoking : Yes / No

2. If yes : Same No as before / Reduced

VII ASSESSMENT OF WOUND HEALING & PAIN AFTER LASER ABLATION

Criteria / Time Period	3rd Day	2 Weeks	1 Month	3 Months
Wound Healing				
Pain in VAS				

VIII PROGNOSIS OF TREATMENT

1. Regression in size of Lesion : Yes / No

2. Formation of New Lesion : Yes / No

3. Malignant Transformation : Yes / No

4. Change in the Nature of Lesion : Yes / No

   If yes, Erythematous / Proliferative

5. Salivary Antioxidant level after 3 months : SOD : _____

   GP : _____

Signature of Trainee : _____ Signature of Guide : _____

Annexure - III

INFORMED CONSENT FORM

Evaluation of efficacy of laser ablation in the management of oral leukoplakia

Name: Age/Sex Op.No: Date:

Address:

I, _____ aged _____ have been informed about my role in the study.

1. I agree to give my personal details like name, age, sex, address, previous dental history & the details required for the study to the best of my knowledge.
2. I will co-operate with the dentist for my intra oral examination & extra oral examination.
3. I will follow the instructions given to me by the doctor during study.
4. I permit the dentist to take blood sample, photos, intraoral radiographs & I accept to undergo bone regenerative procedures as required for the study.
5. If unable to participate into study for reasons unknown, I can withdraw from the study.

In my full consciousness & presence of mind, after understanding all the procedures in my own language, I am willing & give my consent to participate in this study.

Name of the patient: Name of the investigator:

Signature/Thumb impression Signature

Annexure - IV

ஒப்புகை வாக்குமூலம்

..... ஆகிய நான் மேற்கூறிய ஆராய்ச்சி படிப்பின் வழிமுறைகளைத் தெளிவாகப் புரிந்து கொண்டேன். மேலும் நான் இந்த ஆராய்ச்சிப் படிப்புக்கான வழிமுறைகளை மேற்கொள்வதற்கும், அதன் பரிசோதனை முடிவுகளை தெரிந்து கொள்ளவும் முழுமையாக அனுமதிக்கிறேன்.

.....

நோயாளியின் கையொப்பம் தேதி.....

நான் மேற்கூறிய ஆராய்ச்சிப் படிப்பிற்கான விதிமுறைகள் மற்றும் அது குறித்த நோயாளியின் சந்தேகங்களையும் தெளிவாக விளக்கியுள்ளேன்.

.....

மருத்துவரின் கையொப்பம் தேதி.....

Annexure - V

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

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Chairman
Dr. P. PONMURUGAN, Ph.D.,
Prof. & Head Dept. of Biotechnology
KSR College of Technology,
KSR Kalvi Nagar, Tiruchengode.

Member Secretary
Dr. G.S. KUMAR, MDS.,
Principal,
KSR Institute of Dental Science & Research,
KSR Kalvi Nagar, Tiruchengode.

Ref.: 053 /KSRIDSR/EC/2014 **Date : 09.01.2014**

To

Dr.P.Harish Babu,
Postgraduate Student,
Dept. of Oral Medicine & Radiology,
KSR Institute of Dental Science & Research,

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Your dissertational study titled "EVALUATION OF EFFICACY OF LASER ABLATION IN THE MANAGEMENT OF ORAL LEUKOPLAKIA" presented before the ethical committee on 07th Jan. 2014 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary,
(Dr.G.S.Kumar)

PRINCIPAL,
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
THOKKAVADI POST,
TIRUCHENGODE - 637 215.