MORPHOLOGICAL CHANGES IN ORAL MUCOSA OF RABBITS INDUCED BY LIGHT EMITTING DIODE (LED) USED AS DENTAL CURING LIGHT

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ABSTRACT

A dental curing light is a piece of dental tool that is used for the hardening of light cure composites. Many studies have shown these lights induce changes in DNA, mitosis and mitochondria through free radicals production. Light emitting diode (LED) are most commonly used and claim less hazards to the adjacent soft tissues. Current study was therefore designed to observe the morphological changes induced by light emitting diode (LED) as a dental curing light source in oral mucosa of experimental animals.

Fifty rabbits were divided into 5 groups (4 experimental and 5th as a control group). Cervical margin of central incisors of each animal in the experimental groups was exposed 3 times with LED light; duration of each exposure being 40 seconds with a gap of 30 seconds. Punch biopsies were taken after 24 hours, 48 hours, 1week and 2weeks from group 1, 2, 3 and 4 animals respectively.

Results showed ulceration (4%), acanthosis and vascular pathological changes (100%), enlarge (bulbous) rete ridges (97.5%), basal layer vacuolization (85%), acantholysis (27.5%) and atypical mitosis (10%) in all the experimental animals. With passage of time, a significant increase (P=0.000) in frequency of basal cell hyperplasia (90% in group 1 to 100% in group 4) and basal layer atypia (70% in group 1 to 90% in group 4) was observed. While inflammation dropped from 100% in group 1 to 0% in group 4 (P=0.000) due to healing of tissues. Changes were similar to the previous studies except some severe effects like atypical mitosis and basal layer atypia were observed which may be attributed to increase in number of light exposures in our study that is in compliance with the clinical practice in our set up.

These findings may help in creating awareness among the dental practitioners to use dental curing lights with caution keeping appropriate safety measures for the adjacent oral soft tissue in consideration.

Key Words: *Morphological changes, oral mucosa, light emitting diode.*

INTRODUCTION

A dental curing light is a dental tool used for the hardening of light cure composites.[1] Light-emitting diode (LED) was introduced to overcome the problems associated with previous light sources. It provides blue visible light having a spectrum in the range of 450nm to 490nm.[2] Curing light is employed in many aspects of modern dentistry like restorative, preventive and orthodontic dentistry.[3] Usually, composite material is applied in the form of small increments which are cured each time except in very small restorations. In order to get better result of final restoration, low intensity should be used with extended intervals during which polymerization is allowed to continue.[4] When using latest generation of LED units having light intensity of 1,500-2,000 mW/cm2 curing time can be reduced to 20 seconds.[5] In addition to curing the material, this high intensity light can damage nearby tissues i.e. oral mucosa through its biological effects and rise in temperature.[6]

Experimental studies have shown that oral tissues when exposed to curing light, results in a T-cell induced inflammation.[7] Due to the heat generated by the curing

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unit and the exothermic nature of the polymerization process itself, the rise in temperature may cause coagulation of protoplasm, expansion and outflow of fluid from the dentinal tubules, changes in blood vessel structure and tissue necrosis.[8] Angiogenesis is normally a tightly regulated process that rarely occurs in the adult organism. There are certain pathological conditions including cancer, diabetic retinopathy, ischemic cardiovascular and chronic inflammatory diseases associated with aberrant angiogenesis. Angiogenesis is an essential process to supply nutrients, oxygen and growth factors thus supporting cellular function and survival. It involves the complex interaction between endothelial cells, extracellular matrix proteins and soluble factors in plasma.[9] The present study we used rabbits of New Zealand breed, to study the angiogenic changes in oral mucosa with three exposures of the light each of 40sec with a gap of 30sec because morphological features of rabbits are closely similar to human gingival mucosa.

METHODOLOGY

This was an experimental study, conducted in the Experimental (Animal) Research Laboratory and the Department of Morbid Anatomy and Histopathology/ Oral Pathology, University of Health Sciences, Lahore. The sample size of 10 rabbits/ group was calculated by Gertsman formula.[10] A total of 50 rabbits were divided randomly through balloting/lottery method into 5 groups, each consisted of 10 rabbits. One group was control and other four were experimental groups. The study animals were maintained in The Experimental Research Laboratory of University of Health Sciences, Lahore, under standard environment and diet, two weeks before start of experiment.

The Dental Curing Light, LED. B made by Guilin Woodpecker Medical Instrument Co., Ltd. Patent no. CN 200730092316.9 with light output 850mW/cm2-1000mW/cm2 and wave length 420nm to 480nm was used. Cervical margin of central incisors was exposed for 40 seconds, exposure was done 3 time with 30 sec gap between each exposure because composite filling is done in small increments in most of the cases but in earlier studies only one exposure for 40 seconds. Biopsies were taken after 24 hours, 48 hours, 1week and 2weeks from group 1, 2, 3 and 4 animals respectively. The specimens from the specified area i.e. oral mucosa adjacent to the maxillary central incisor were obtained after anesthetizing the animals. Representative tissue sections were taken and tissue specimens were fixed in 10% formalin and processed. Paraffin embedded blocks were made after taking these through ascending grades of ethanol. The sections 3-4 micro meter were cut using microtome and stained using Haemotoxylin and eosin stain and submitted for microscopic examination.

Statistical Analysis

The data were entered and analyzed using SPSS 18.0. Mean ± SD was given for quantitative variables like weight and the frequencies along with percentages were given for qualitative variables like gender and morphological changes. Pearson Chi Square and Fisher exact test were applied to observe associations between groups and morphological changes. A P-value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of n=50 rabbits including females = 11(22%) and males = 39(78%), with mean weight 1.765±0.1767 kgs (Range: 1.5→2.0 kgs) were included. As regards pathological characteristics observed in the experimental animals, gross morphological features like consistency and no areas of necrosis were observed in both control and experimental animals while considering the colour of tissue it appeared as pink, reddish pink and reddish. The pink colour was present in 50.0%, 30.0%, 100.0% and 60.0% cases of group 1, 2, 3 and 4 respectively. Reddish pink colour was present in 20% cases of group 1 and 40% cases of both group 2 and 4 while group 3 did not show reddish pink color. Reddish colour was seen only in group 1 and 2 in 30% cases. Overall, the following microscopic changes were noted.

Within two weeks duration, a significant increase (P=0.000) in basal cell hyperplasia (90% in group1 to 100% in group4) was also observed. Acute inflammation showing predominantly neutrophilic infiltrate with macrophages was observed in 100% each of the animals in group1 and group 2 while only 30% and none of the animals in group 3 and 4 respectively. Regarding the vascular changes, angiogenesis and thickened vessel walls were found in 100% (Fig 3) while vascular congestion and dilatation was seen in 82.5% of the experimental animals.

DISCUSSION

This study was done to observe the histological changes in oral mucosa of rabbits induced by light emitting diode (LED) used as dental curing light. We used rabbits of New Zealand breed, as morphological features of rabbits are closely similar to human gingival mucosa as well as ease of their availability.[11]

Pathological variables were studied and results were compiled. As regards the microscopic changes,

ulceration[12] was reported in the present study while none of other studies in the literature described such a change. It is usually believed that visible light sources (LEDs) are non-mutagenic but they can incite thermal energy in biological tissues which can cause burns and colour changes as observed in experimental animals (from pink to red). In the present study Acanthosis, the hyperplasia of prickle cell layer was observed in all

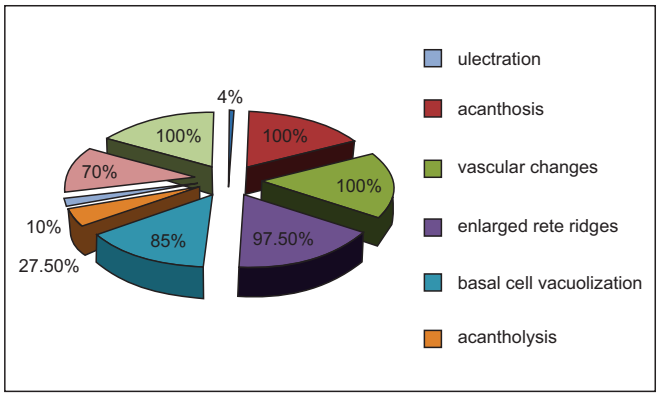

Fig 1: This figure shows distribution of different histological changes among n=40 experimental animals

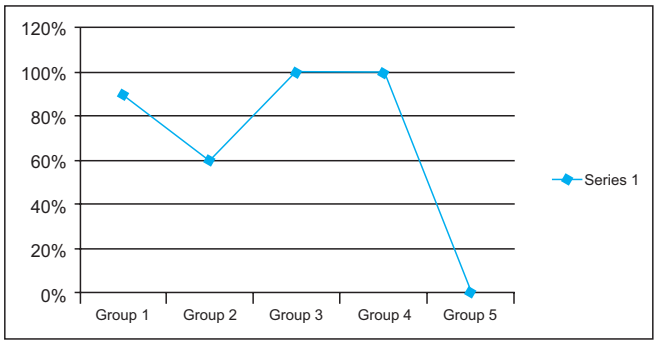

Fig 2: This figure shows frequency of basal cell hyperplasia in relation to time duration among different groups. Note that increase in frequency from group1 (90%) to group4 (100%) of experimental animals

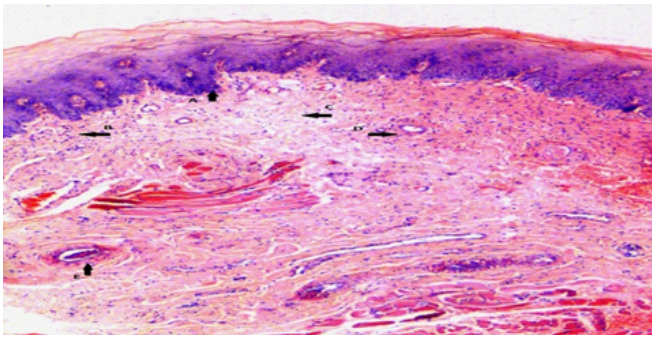

Fig 3: Photomicrograph of gingival mucosa of rabbit in experimental group 2 showing basal layer atypia (arrow A), angiogenesis (arrow B), stromal edema (arrow C) perivascular acute and chronic inflammatory cells (arrow D) and vessel wall thickness (arrow E). (H&E, 10 ×10x)

experimental groups (100%). Nearly all precancerous lesions (leukoplakia, erythroplakia and lichen planus) in their histology shows acanthotic change but it is reactive in nature in most of the cases.[13] Similar changes were noted in studies performed by Mohamed and Rita in 1997 in Egypt and Swad in 2009 in Iraq, however, rats and rabbits were used as experimental animals respectively.[14,15] In both studies a single light exposure of 40 seconds was applied. On contrary, a progressive decrease in epithelial thickness was observed in a study carried out by Murdiastuti and associates in 2010 in Indonesia.[16] Acantholysis is the loss of intercellular bridges of cells of spinosum layer which can result in the formation of blisters and ulcers in oral mucosa.[17] It was observed in 27.5% of experimental groups with no such change in control group (P=0.000). Mohamed and Rita documented similar change in oral mucosa in their experimental groups in their study performed in Egypt in 1997.[14]

Mittal and co-authors reported that when skin is exposed to UV radiations, infiltrating CDl lb+ inflammatory cells result in oxidative stress which overcome normal anti oxidative mechanism of skin leading to basal cell vacuolization observed in phototherapy.[18] Same mechanism is involved in visible light through LED. In the present study, basal cell vacuolization was observed 80% with 100% each of group 1 and 2 while in 90% and 50% of group 3 and 4 respectively of experimental animals while no such change among control group was noted (P=0.000). Mohamed and Rita also documented such change.[14] In present study basal cell hyperplasia was found but none of other studies related to our work described such a change. Basal layer atypia characterized by change in the basal cells showing pleomorphism, altered N/C ratio, prominent nucleoli, and increase in typical mitotic figures[19] was reported in the present study. No study in the literature related to LED effects on oral mucosa described such a change. Atypical mitosis includes tripolar, multipolar and ring shaped mitotic bodies/ figures within the nucleus as described by Rubio.[20] In the present study it was observed predominantly in basal and lower 2/3rd of the keratinocytes of the mucosa of 10% animals of the total 40 experimental animals. While none of the control group animal depicted such change (P=0.783). Although finding is statistically not significant but increase in mitotic rate and presence of these atypical mitosis might be a significant pathological change if it persists even after cessation of stimulus. None of the studies done on Light curing effects on oral mucosa described such a change which might be

related because of a single exposure to light whereas in our study three exposures were done which is in compliance with the clinical practice on human patients in our clinical set up.

A part from epithelial changes, significant stromal alterations were also observed. Stromal oedema which was observed from 90% to 100% experimental animals it may signify a healing process. None of the control group animals showed this change (P=0.000). This finding is consistent with the results of the studies done by Mohamed and Rita as well as Swad.[14,15]

Visible light from curing unit gives thermal and photo dynamic stimuli resulting in an acute inflammatory response.[21] In present study acute inflammation with predominantly neutrophils and macrophages was observed in 100% animals in each in group 1 and 2 while in 30% and 0% of experimental animals in group 3 and 4 respectively. Results shows that in early 48 hours (group 1 and 2) there is maximum inflammatory response which decrease to 30% after 1week (group 3) and 0% after two weeks (group 4). None of the animals in the control group depicted such change (P=0.000). Murdiastuti and associates in 2010 at Indonesia reported similar finding in his study.[16] Swad in 2009 at Iraq aslo observed inflammation in his study.[15] Chronic inflammatory infiltrate predominantly lymphoplasmacytic and macrophages with plump fibroblast was observed in 100% of all groups of experimental animals, while none of the animals depicted such change in control group (P=0.000). Visible light from curing unit acts as irritant and result in vessel congestion and dilatation which results in an increase blood flow to that area helping in inflammatory process and to overcome oxidative stress. In present study vessel wall congestion and dilatation was observed 82.5% with 100% animals each in group 1, 2 and 4 while in 30% in group 3 animals. Findings are same in group 1, 2 and 4 (100%) while drop (30%) in group 3 may be due to host response. When compared to control group, none of the animal depicted such a change (P=0.000). Vessel wall thickness increased due to increased protein deposition (hyalinization) and also formation of neo intima. It's a healing response of vessel to any stimuli.[22] It was in response to increasing exposure to dental curing light (LED) and reported in 100% animals of all the experimental groups, while none of control animals depicted such a change (P=0.000).

CONCLUSION

To date, much data is available on the efficacy of dental curing light and composite material but little focus has been given to the hazardous effects of curing light on the oral mucosa adjacent to the tooth being irradiated. Results of the present study were similar to the previous studies except some severe effects like atypical mitosis, basal layer atypia and basal cell hyperplasia are exclusively reported in the present study, which may be attributed to increase in number of exposure in the present study, compliant with the usual clinical procedure.

Stromal inflammation and vascular pathological changes including angiogenesis were also significantly observed in all experimental group animals. To our knowledge no such study is done in Pakistan as well as no relevant data in the previous literature describing the actual statistics related to the histological changes in experimental animals exposed to LED has been found. So, the present study not only is an important addition in terms of reporting the statistically significant histological findings in oral mucosa after exposure to LED light source but also may help in creating awareness among dental practitioner to use dental curing light (LED) with caution maintaining protective measures for the adjacent oral soft tissue in consideration.

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