Umm Al-Qura Univ. Medical J. Vol. 3, No.2, pp. 98-107 (2012)
01-06 © The University Press

UQU Medical Journal

Original Article

Clinical And Ultrastructural Evaluation Of Topical Application Of Vitamin E In Chronic Gingivitis Associated With Removable Partial Dentures

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التقييم السريري و الميكروسكوبي للتأثير الموضعي لفيتامين هـ في علاج التهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة.

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الملخص العربي:

الهدف : تهدف الدراسة لتقييم التأثير الموضعي لفيتامين هـ كمضاد للأكسدة في علاج التهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة.
الطريقة : تم تطبيق هذا البحث على أربعه عشر موضعا لالتهاب اللثة المزمن في عشرة أشخاص ممن يستخدمون الأطقم الجزئية المتحركة و يعانون من التهاب اللثة تم تقسيمهم كالأتي: المجموعه الأولى تم علاجها بازاله الرواسب الجيرية و الكحت اللاجراحى مع أضافه فيتامين هـ كماده مضادة للأكسدة على هيئه زيت موضعي أما المجموعة الثانية فقد تمت معالجتها بازاله الرواسب الجيرية و الكحت اللاجراحى فقط كما تم تقييم المجموعتين اكلينيكيا و ميكروسكوبيا باستخدام المجهر الألكترونى .
النتائج : دلت نتائج هذا البحث على أن استخدام فيتامين هـ قد أدت إلى تحسن ذو دلالة أحصائية فى حالة اللثة أكلينيكيا من حيث درجة قياس التهاب اللثة و معامل الرواسب الجيرية. وقوى هذه النتائج الفحص تحت المجهري لأنسجة اللثة حيث أدى استخدام المادة المستخدمة فى البحث الي زيادة ترابط خلايا النسيج الخلوى للثة مع تحسن فى أغشية الأنوية و الميتوكوندريا.
الخلاصة: بناء علي النتائج السابقة فانه يوصى بإضافة المواد المضادة للأكسدة مثل فيتامين هـ إلى العلاج التقليدي لالتهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة حيث انه يسرع في تحسين حالتها إكلينيكيا ويمنع من تطور المرض .

ABSTRACT

Objectives: Objectives: To evaluate the clinical and biological effects of topical application of vitamin E as an antioxidant therapy in chronic gingivitis associated with removable partial dentures.
Methods: Fourteen chronic gingivitis involved sites were enrolled for this study, they were divided into two groups. Group 1: comprised seven chronic gingivitis involved sites managed by mechanical debridement with topical application of vitamin E , and Group II: comprised seven chronic gingivitis involved sites managed by mechanical debridement only. Moreover, a biopsy(1x1mm) was taken from the interdental papillae of the selected sites of both groups to be examined using the transmission electron microscope.
Results: Clinical results showed significant improvement in clinical parameters (plaque index and gingival index) in both studied groups. The magnitude of improvement was in favor to group I. A statistically significant difference was observed between group I and group II only in relation to the gingival index. The histological results obtained from the present study revealed that: In group II, abnormal changes in the gingival keratinocytes and fibroblasts especially the nuclear membrane, desmosomal junction, and mitochondria. While in group I marked improvement was observed following the topical application of antioxidant therapy.
Conclusion: The topical application of vitamin E improves the gingival tissues clinically and ultra structurally and it is recommended to be used as an adjunctive treatment in chronic gingivitis in patients wearing removable partial dentures.

Keywords*: dentures, gingivitis, vitamin E, antioxidants.*

INTRODUCTION

Chronic gingivitis is an inflammatory disorder caused by substances derived from microbial plaque accumulating at or near the gingival sulcus; all other suspected local and systemic etiologic factors either enhance plaque accumulation or retention, or enhance the susceptibility of the gingival tissue to microbial attack.[1]

It was suggested that insertion of removable partial dentures creates the potential for quantitative and qualitative changes of plaque formation on the remaining teeth that is representative by proliferation of spiral organisms. Thereby there is an increased risk for development of gingivitis and periodontitis.[2,3]

Multiple molecular players are involved in gingivitis and periodontitis, among them is reactive oxygen species(ROS) which is postulated to have a role in both the bacterial and host mediated pathway of tissue damage[4].

ROS is the end result of the reduction of molecular oxygen which is required for all mammalian cells to obtain energy. This reduction process is accompanied by a large free energy release that gives rise to free radicals and/or ROS [5].

ROS has been adopted to include molecules such as hydrogen peroxide, hypochlorous acid and single oxygen. They cause tissue damage by a variety of mechanisms including DNA damage, and lipid peroxidation. Moreover it exerts protein damage of gingival hyaluronic acid and proteoglycans, it also stimulates pro-inflammatory cytokine release by monocytes and macrophages[6].

Whilst most ROS have extremely short lives, they can cause substantial tissue damage. It is therefore not surprising that the body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants or ROS as soon as they form, or to repair damage caused by ROS in vivo[7].

Antioxidant defense mechanisms are located in both water or aqueous part of our body. Among the major antioxidants are vitamin E, ubiquinol and various carotenoids derived from dietary sources[8].

Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities. Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. It was investigated that vitamin E might help prevent or delay the chronic diseases associated with free radicals by neutralizing free radicals that cause oxidative cellular damage.[9]

Moreover, vitamin E exhibits anti-inflammatory properties which may limit inflammation-induced tissue destruction. As an antioxidant, vitamin E may protect lysosome membranes leading to a decrease in histamine and serotonin from mast cells during inflammation. [10]

Studies [11-12] indicated that in chronic gingivitis and periodontitis ROS overproduction can also be induced by periodontal pathogens that may induce collagen and periodontal cell breakdown . It was also suggested that when ROS are scavenged by antioxidants there can be a reduction of collagen degradation.[13] .

In another study it was found also that the ROS not only affect the progression of chronic gingivitis, but they are also proved to increase the severity of inflammation in peri-implant tissues.[14]

Recently, in 2008,[15] the relationship between ROS and apoptosis in normal human keratinocytes and fibroblasts was studied ultrastructurally and it was reported that they can induce apoptotic cell death in form of chromatin condensation, plasma membrane blebbing and rounding up of cells in primary normal keratinocytes and fibroblast.

Thus the role of antioxidants as a defense agent against the free radicals released by the mitochondria is no longer a point of debate; however, further studies are needed to detect their clinical and biological effect on the gingival and periodontal tissues in chronic periodontitis.

The aim of the present study was to evaluate the clinical and ultrastructural effect of the topical application of vitamin E in gingival tissue of chronic gingivitis patients with removable partial dentures.

MATERIAL AND METHODS

I. Patients' selection

A total of fourteen matched chronic gingivitis sites from ten patients were chosen from the outpatient clinic of Oral Medicine and Periodontology department, Faculty of Dentistry, Alexandria University, whose age ranged from 32-56 years with a mean age of42 years. Informed consent was obtained from each subject prior to the study.

The patients were chosen on the basis of:

1- Wearing removable partial dentures with at least one year with chronic gingivitis related to the abutment teeth. [16]

2- Systemically healthy, nonsmoker males and with no history of any drug therapy in the 3 months preceding time of research.

II. Materials

Vitamin E antioxidant 400mg capsules *was used in the present study.

III. Methods

Each patient was subjected to a thorough clinical dental examination including

plaque index PI [17,18], gingival index GI. [19]

After base line examination all patients were given oral hygiene instructions and they were randomly divided into two groups:

Group 1: Mechanical debridement consisting of supragingival scaling was performed using hand instruments and ultrasonic scalers in addition to topical application of400mg vitamin E * twice daily for a period of six weeks.

Group II: Managed only by mechanical debridement. All clinical parameters were taken prior to treatment and 6weeks following treatment. All patients were given oral hygiene instructions and placed on strict maintenance recall visits during which oral hygiene was reviewed. The data was collected, tabulated and statistically analyzed by Mann-Whitney test.

Histological study:

A biopsy(1x1mm) was taken from the interdental papillae of the selected sites of both the test and control sites fixed in 2% glutaraldehyde solution and 2% formaldehyde and immediately in 5M sodium phosphate buffered at pH 7.3. Then the tissues were cut into pieces `1-2mm^2, rinsed in cacodylate buffer for one hour and post fixed for two hours in 1% osmium tetroxide buffered with 0.15M sodium phosphate at pH7.3. After this post fixation, the tissues were rinsed in buffer, dehydrated in ethanol and embedded in Epon Araldite.[20]

Semi thin sections were stained with methylene blue and examined with the light microscope. Ultrathin sections were cut and stained with alcoholic uranyl acetate and lead citrate. And then examined with Jeol electron microscope100cx.[21].

RESULTS

Clinical Results

Statistically significant improvement was observed in PI in both studied groups at the six weeks follow up period when compared to the base line values.

On comparing both treated groups, no significant difference was found in PI at various periods of follow up (Table I).

Table (1): Comparison between group I and group II in the different stages according to GI , PI.

		Base line		6 weeks	
		G II	**G I**	**G II**	**G I**
GI	**Mean ± SD**	2.29 ± 0.76	2.29 ± 0.76	1.43 ± 0.79	0.29 ± 0.49
	U (p)	24.500 (1.000)		6.500* (0.014)	
	Z (p)			1.604 (0.109)	2.392* (0.017)
PI	**Mean ± SD**	2.14 ± 0.69	2.00 ± 0.82	0.71 ± 0.76	1.00 ± 0.82
	U (p)	22.000 (0.728)		19.500 (0.493)	
	Z (p)			2.232* (0.026)	2.333* (0.020)

U: Mann-Whitney test between G I and G II in the different stages

Z : Z for Wilcoxon signed ranks test between base line and the other stages

* : Statistically significant at $p \leq 0.05$

Table (2):Comparison between the two studied groups according to % of change between base line and 6 weeks

	G II	**G I**	**U (p)**
GI	28.6 ± 40.50	88.10 ± 20.9	7.500* (0.025)
PI	35.80 ± 39.30	57.10 ± 61.90	23.000 (0.842)

U: Mann-Whitney test between G I and G II in the different stages

* : Statistically significant at $p \leq 0.05$

Histological Results

Group I

The gingival epithelium

The basal cells contained large nuclei; the nuclei were bound by evenly contoured nuclear membrane. The cell organelles were found around the nuclei. The basal lamina was distinct.

The keratinous cells were densely packed with tonofibrils, rounded empty spaces of various diameters occurred within the cytoplasm (fig.1,2)

The lamina propria

There was a subsidence of the inflammatory reaction in the connective tissue.

The fibroblast was found to be a typical secretory cell with fusiform nucleus, a abundant rough endoplasmic reticulum, mitochondria and numerous membrane bound vesicles located near the cell membrane. (fig. 3).

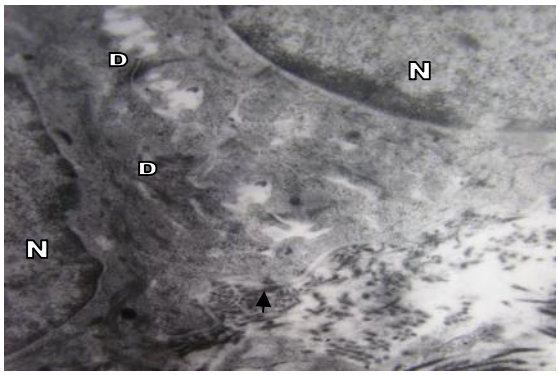

Fig (1) basal cells with more or less rounded nucleus (N). Note many desmosomal junctions (D) there is thick basal lamina (arrow)
X10000

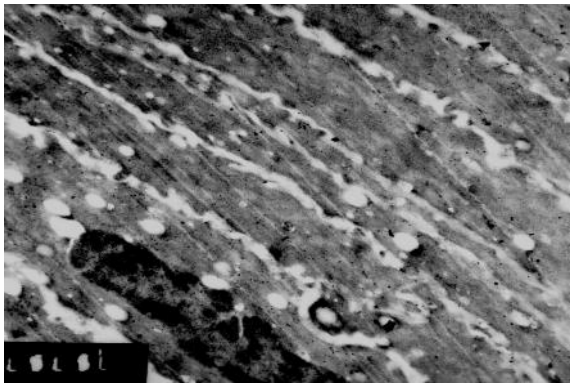

Fig (2) keratinous layer with dense tonofibrills. Granular cell (G). Note the interdigitation between the cell membranes. x 7500

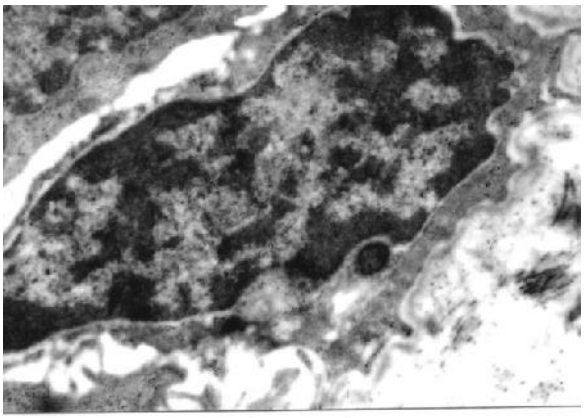

Fig (3) fibroblast in the Lamina propria shows well developed nucleus.& L.S.x14000

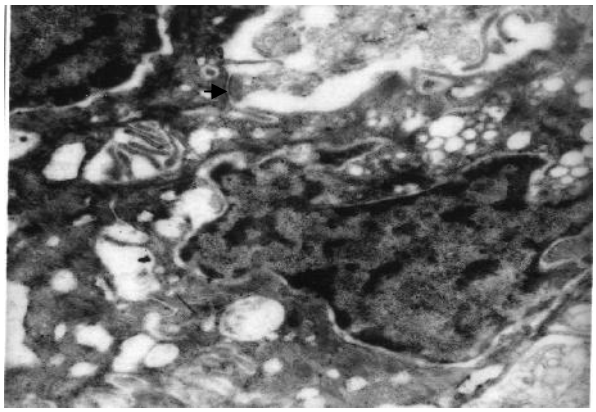

Fig (4) the basal cells with few desmosomal junction (D) and wide intercellular spaces (IS)filled with amorphous substance. The nucleus shows irregular outline. note the vacuoles (V)in the cytoplasm. X 10000.

Group II

The gingival epithelium

-The nuclei of the basal cells showed irregular outline with vacuolated cytoplasm, few desmosomal junctions could be seen in between the cells. The intercellular spaces were wide and filled with amorphous substance. The basal lamina was indistinct.

-The keratinous layer showed wide intercellular spaces filled with amorphous substance, no desmosomal junction was noticed. (fig.4,5)

The lamina propria

-The fibroblasts were found to show atypical appearance with irregular outline of the nuclei and vacuolated cytoplasm.

-The mitochondria were swollen and have floculant appearance. Many inflammatory cells were found in the lamina propria. The macrophages showed indented nucleus and many phagosomes in their cytoplasm (fig.6,7).

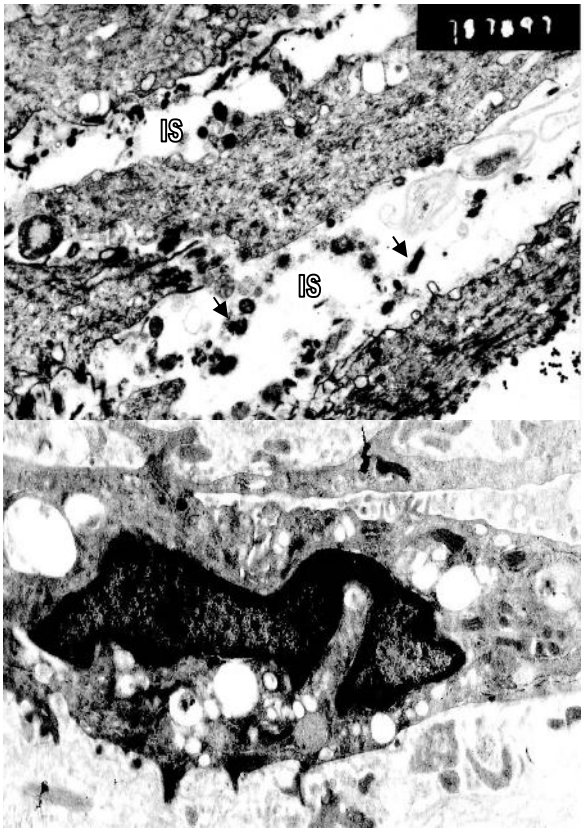

Fig (6) fibroblast in the lamina propria with irregular outline of the nucleus. The cytoplasm shows many vacuoles& Mitochondria X 7500.

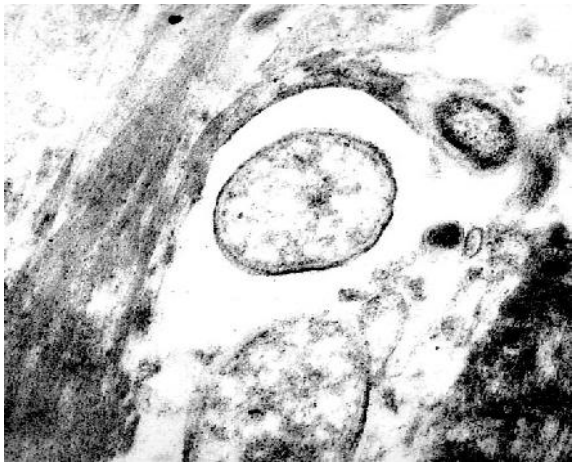

Fig (7) mitochondria with a floculant appearance (M). X 15000.

DISCUSSION

There has recently great interest in antioxidants for the treatment and prevention of disease. Vitamin E, as an important antioxidant, has received much research attention in the medical literature in the last several years[9,22].

A removable partial denture is a common treatment available for restoration of partially edentulous ridges. Many investigators have studied the effect of RPDs on gingival health. [23,24]Orr et al [25] reported an increase in gingival index after 21 days of constructing acrylic resin base plate, moreover, longitudinal studies indicate that RPDs have been associated with increased gingivitis, periodontitis, and abutment mobility.[26]

Studies [27,28] indicated that the reaction of the host inflammatory and immune responses to pathogenic species within the periodontal environment can generate reactive oxygen species within the adjacent tissues. Protection against such species is provided by antioxidants, many of which are released locally at sites of infection by inflammatory cells such as PMNs. It is likely that the local antioxidant system act in concert of total antioxidants capacity in gingival crevicular fluid and deficiencies in these systems could place individuals at increased risk of destructive periodontal diseases [29].In the current study, the antioxidant vitamin E was used topically as an adjunctive therapy to traditional nonsurgical debridement in sites with chronic gingivitis in patients with RPD. This therapeutic management was evaluated clinically and ultra structurally and compared to non-surgical debridement alone. The selected individuals of the present study were all systemically healthy and non-smokers. Studies indicated that smoking increases gingival inflammation in periodontal tissue of smoker periodontal patients that may occur due to the effect of smoking on the defense mechanism of the gingiva against free radicals causing exaggerated inflammatory response [30].The clinical results of the present study showed that plaque index exhibited reduced scores during the course of treatment and did not present statistically significant differences

between the two groups at various study periods. It appears that the reduced plaque scores was accomplished by the continuous reinforcement of home oral and denture hygiene measures and the professional dental care program during the whole study period. The results of the present study revealed also a statistically significant differences between group I and group II in relation to the gingival index at 6 weeks post treatment period. The significant decrease in GI may be attributed to the low PI, the increase of antioxidant level in gingival and periodontal environment and the effect of vitamin E on microbial environment in group I following the application of vitamin E.Okada et al [31] recommended that treatment with oral administration of antioxidant may restore the immune function in chronic periodontitis where the aggression of bacterial plaque is frequently enhanced by unfavorable regional defects in immune system. In the current study, the histological results of group II revealed wide intercellular spaces filled with amorphous substances, altered few desmosomal attachment and ill formed tonofilaments between the gingival keratinocytes. The fibroblasts showed incomplete nuclear membranes, and mitochondrial alteration with many autophagic vacuoles filled with collagen fibers indicating high rate of fibrin turn over. These histological features could explain the increased inflammatory condition as manifested by increase GI when compared with group I. On the other hand, the histological results obtained from group I revealed normal appearance of the basal cell with gingival epithelial cells packed with many tonofilaments, small intercellular spaces with many desmosomal junction. This was manifested clinically by firm shrinkage gingiva with no exudates (significant decrease in GI). The lamina propria showed many fibroblasts in the active stage of protein synthesis denoting the subsidence of inflammatory condition and the fibrotic appearance of the gingiva following application of vitamin E. Our result are in accordance to Sobaniec et al [32] who demonstrated destruction of alveolodental ligament in the of experimental ligature-induced periodontitis in rats . The authors suggested that these results may occur due to the decrease of basic antioxidant enzymes activity with simultaneous increase of the final products of lipid peroxidation. Moreover, Wadleigh et al [33] found that vitamin E is effective in the treatment of gingivostomatitis. A randomized, double blind placebo-controlled study was done to determine whether topical Vitamin E would be effective in healing mucositis. A total of eighteen adult patients receiving chemotherapy for various types of malignancies were included in this study. Six of the nine patients who received Vitamin E had complete resolution of their oral lesions compared with only one out of nine patients who received the placebo (p = .025). These results suggest that Vitamin E may be effective therapy in treatment of gingivostomatitis. On contrary to our results, Parish et al[34] found no beneficial effects from the therapeutic use of vitamin E to combat periodontitis. The authors conducted a study on the effect of vitamin E on the course of periodontitis in thirty-six adult male albino rats. The rats were divided into three groups of twelve and placed on test diets that either was deficient in, or contained adequate and high amounts of, vitamin E. A local irritant in the form of a stainless steel wire was placed around the maxillary left second molar of each animal as a collector of plaque and debris. Migration of the epithelial attachment, alveolar bone level, and numbers of inflammatory round cells were then evaluated on both sides of the maxilla. The results of this experiment indicate that a deficiency of vitamin E does not cause increased destruction of the periodontium in the presence of periodontitis. Moreover, no beneficial effects from the therapeutic use of vitamin E to combat periodontitis was found. The importance of antioxidants in

inflammatory tissues has been studied in vivo[35]. Degradation of collagen powder by experimental granulation tissue induced by cellulose sponges in the rat was monitored as the radioactivity excreted in urine. By administering pharmacological doses of both vitamin E and selenium subcutaneously and by injection into sponges implanted subcutaneously, this breakdown of collagen was reduced. Injections in the sponges also arrested the maturation of the granulation tissue. The results revealed that vitamin E and selenium are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells. It is therefore suggested that these radicals may play a role in the collagen destruction by granulation tissues, as in periodontal diseases.

CONCLUSIONS

1. The topical application of vitamin E in adjunct with supragingival debridement further improved GI and PI in chronic gingivitis associated with RPD.
2. The topical application of vitamin E might enhance resistance of gingival tissue destruction as manifested by the histological evidence.
3. Vitamin E has proved to be an easy method for topical application of antioxidant therapy.

RECOMMENDATIONS

1. Further studies are indicated in order to evaluate the use of antioxidant therapy in periodontitis.
2. The involvement of antioxidant material in a carrier system is recommended in order to achieve an effective duration and drug concentration of therapy.
3. Routine supplementary use of antioxidant therapy is recommended as therapeutic management or protection from destructive periodontal diseases.

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