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ORIGINAL ARTICLE

Evaluation of Effect of Astringent on Oral Mucosa as a Non-surgical Preprosthetic Treatment Modality in Edentulous Patients: An In Vivo Study

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Abstract Preprosthetic treatments are advocated in edentulous patients to enhance the denture bearing areas for good denture support. Most of the times the preprosthetic treatments are considered only in a surgical way. Ideally every edentulous patient undergoing complete denture treatment needs a non-surgical preprosthetic treatment. So that, the denture bearing area will be properly prepared before the denture construction. The present study was conducted on thirty completely edentulous male patients who had visited to our Institute for the treatment. Each patient was asked to massage with astringent gel on the denture bearing mucosa over a 4 weeks period. Exfoliative cytology was used to collect the surface cells from the palatal mucosa. First scrape was taken before the stimulation treatment was started. The second and third scrape was taken after the stimulation treatment with astringent gel for each patient. In this way total 90 scrapes were made and the each smear was stained with the Papanicolaou's technique to examine under light microscope. About 100 cells were counted from each stained smear. The number of parabasal cells, intermediate cells and superficial cells were recorded to calculate the degree of keratinization. Statistical analysis was performed. A significant difference ($p < 0.001$) in keratinization levels was found. The mean percentage of superficial cells before the stimulation treatment was 79.80 %; this percentage was gradually increased to 84.60 and 90.57 % after the 2 and 4 weeks period of stimulation treatment respectively with astringent gel.

Keywords Preprosthetic treatment · Non-surgical · Astringent · Exfoliative cytology · Keratinization · Denture bearing areas

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Introduction

In this era of preventive dentistry and sophisticated methods of replacing teeth, such as with dental implants, conventional complete dentures still remain a viable method of treatment for many patients. One critical aspect of complete denture treatment, which is often confusing and poorly understood, is the proper preparation of the denture bearing areas. Preparation of the denture bearing areas involves surgical or non-surgical approach depending on the needs of the patient. In many instances both forms of treatment are needed to return the denture-bearing tissues to a state of optimal health and form [1].

The success of removable prosthesis depends on the health of denture bearing mucosa. A well keratinized healthy mucosa is desirable for a complete denture. As the life expectancy of human life is increasing; it is our prime duty to extend the complete denture service over many years of an individual's life. The results of many studies done to examine the denture bearing mucosa are found to be contradictory. Some studies suggested that; the denture bearing epithelium becomes more keratinized while others found that there is not only a quantitative reduction of keratinization but also acanthosis as the time passes [2–9]. This will ultimately affect the service of complete denture.

So to keep the denture bearing mucosa in a good physiologic condition, this study was conducted to evaluate the effect of astringent on edentulous mucosa. The tissue sampling was done by exofoliative cytology technique as it has been proved more appropriate for examining the keratinization of the oral mucosa [10].

Materials and Methods

An in vivo prospective study was conducted on thirty completely edentulous male patients who had reported to the Department of Prosthodontics, VSPM's Dental College & Research Centre, Nagpur. Ethical approval was obtained from Institutional Ethics Committee VSPM's Academy of Higher Education.

The Inclusion and Exclusion Criteria for the Selection of Patient

(a) Only male patients between the age group of 40–60 years were chosen. Patients with higher age were not selected to avoid the factor of senile tissue changes [11, 12]. Female patients were not included since changes in the oral mucous membrane and its association shown with menstrual cycle, pregnancy and menopause has been a subject of controversy [13–15].
(b) Habits like smoking, tobacco and betel chewing have also been shown to affect the keratinization of the oral epithelium [16–18] and therefore only individuals free from such habits were selected.
(c) Patients suffering from systemic disorders of long duration or even of the recent past were excluded.
(d) Patients who have completed their total extraction treatment recently and wanted to have a complete denture treatment were selected (old denture wearers were rejected).

The Collection of Cellular Material and Preparation of Smears on Slides

A portion of the hard palate immediately posterior to the rugae zone was selected as the site for obtaining cellular material from the surface of the oral mucosa. The corrugated nature of the surface in the rugae zone and the presence of mucous secretions in the posterior half of the hard palate would make these two areas less suitable for obtaining a smear. The material was obtained by scrapping with wooden spatula.

First scrape was taken from the palatal mucosa before the stimulation treatment was undertaken. The patients were provided with the astringent gel (Gumex by Pharmadent remedies Pvt Ltd in which the main ingredient is tannic acid) and were asked to massage with the help of index finger on the denture bearing areas for three times a day for 10 min. A usage sheet was handed over to each patient and they were asked to tick that sheet when they massaged i.e. three times a day. They were recalled after 2 weeks for the second scrape and after 4 weeks for the third scrape from same area. The usage sheet was checked before taking the scrape. The sheets were collected back after the third scrape.

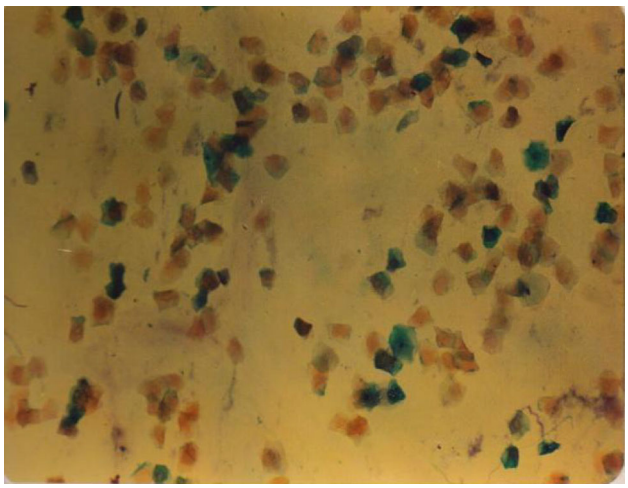

Fig. 1 PAP stained scrape cytological smear before stimulation with astringent

Before intervention	After intervention	
	After 2 weeks	After 4 weeks
First scrape	Second scrape	Third scrape

In this manner a total of 90 smears, three for each of the 30 patients were prepared for the microscopic examination after staining with Papanicolaou's technique.

Interpretation

The slides were studied under the low power (10 × 10) objective lens of a microscope. The field selected for counting the cells was the one where the cells were numerous but not clumped i.e. where the individual cells were distinguishable.

Depending on the morphology and coloring of cells, total numbers of 100 cells were counted for each slide from the selected field. All the 100 counted cells were divided into the following three groups.

I. Parabasal cells — green staining cells.
II. Intermediate cells — blue staining cells.

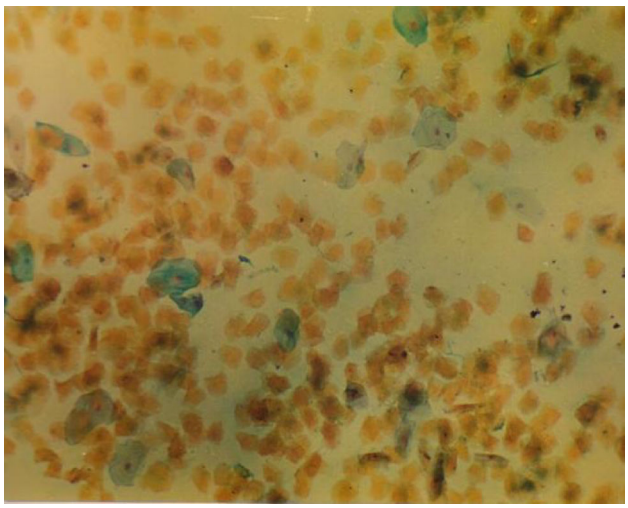

Fig. 2 PAP stained scrape cytological smear after 2 weeks of massage with astringent

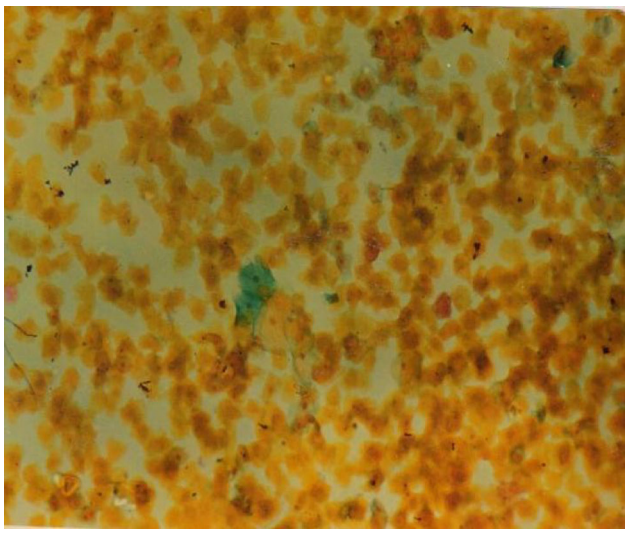

Fig. 3 PAP stained scrape cytological smear after 4 weeks of massage with astringent which shows increase in hyperkeratinized cells

III. Superficial (hyperkeratinized)—orange staining cells.

Although these cells can be further divided into two groups depending on the morphology of nuclei, it was opined by the pathologists that it was very difficult even for an experienced pathologist to differentiate between normal and pyknotic nucleus. Since this differentiation was not essential for the purpose of this study, this was not attempted.

Statistical analysis was carried out using SPSS software.

Table 1 Descriptive statistics of number of basal cells

Duration	Mean	SD	N
Baseline	13.30	2.307	30
After 2 weeks	8.90	1.494	30
After 4 weeks	6.33	1.788	30

Repeated ANOVA measure is used F = 2,038, $p < 0.001$

Table 2 Descriptive statistics of number of intermediate cells

Duration	Mean	SD	N
Baseline	6.77	1.755	30
After 2 weeks	6.50	1.383	30
After 4 weeks	3.10	0.885	30

Repeated ANOVA measure is used F = 1,287, $p < 0.001$

Table 3 Descriptive statistics of number of superficial (hyperkeratinised) cells

Duration	Mean	SD	N
Baseline	79.80	3.067	30
After 2 weeks	84.60	2.527	30
After 4 weeks	90.57	2.402	30

Repeated ANOVA measure is used F = 82,568, $p < 0.001$

N = sample size

Observation and Results

After 2 weeks of stimulation treatment; there was a moderate increase in the number of hyperkeratinized cells as compared to the first scrape as shown in Figs. 1 and 2. After 4 weeks the count had significantly increased as compared to the first scrape as shown in Figs. 1 and 3.

During the 4 weeks period of stimulation treatment; there was a statistically significant decrease in the number of basal cells as shown in Table 1. There was a statistically significant decrease in the number of intermediate cells as shown in Table 2. On the contrary, in this period there was a statistically significant increase in the number of superficial cells (hyperkeratinized) cells as shown in Table 3.

All these observations revealed a gradual increase in keratinization of cells of edentulous mucosa after 2 and 4 week's treatment of astringent gel. This finding was found to be statistically significant.

Discussion

The prosthetic therapy with complete dentures realizes a direct contact between the prosthesis and the mucosal layer [19]. The oral mucous membrane coming in intimate

contact with complete dentures is highly keratinized [20, 21]. This keratinized stratified squamous epithelium undergoes continual replacement by an orderly process involving cell migration and differentiation through four discrete morphologic stages of development. The superficial keratin layer of mucosa is generally thought to be a protective barrier.

A number of studies had been done to assess the keratinization of mucosa under the complete denture. Unfortunately, there remains a controversy within the literature. Some of the studies based on tissue biopsies [22] found increased keratinization [4, 22], while other found a decrease in keratinization [3, 5]. Also some cytological investigations [23] found increased keratinization, while other found decreased in keratinization [24–26].

The maintenance of health of the soft tissue beneath a denture base should be of primary importance for the success of prosthetic treatment [10]. Better keratinization represents a better functional adaptation of the mouth to an artificial appliance [7]. For that denture bearing mucosa should be properly prepared before the denture construction. There are various pre-prosthetic treatment modalities. Most of them include surgical intervention. The purpose of this study is to put forth a non-surgical method of pre-prosthetic treatment; that is by stimulating edentulous mucosa by astringent gum massage. Cytologic techniques have been used for the study of keratinization of oral mucosa as early as 1940 by Weinmann and others [27].

Massage of the denture bearing area in completely edentulous patients has been recommended for problems of ridge soreness accompanying the use of dentures. Kapur and Shklar had undertaken one study to determine the effect of stimulation with an automatic toothbrush on the edentulous ridge and gingivae, where they found increased keratinization of the edentulous mucosa and gingivae [28].

The effect of astringents on oral mucosa was also studied both in dentulous and edentulous patients [29, 30]. Astringents are the substances that precipitate proteins, but do not penetrate cells, thus affecting the superficial layer of mucosa only. They toughen the surface by making it mechanically stronger and decrease exudation. The word "astringent" derives from the Latin word *adstringere*, meaning to "bind fast". They have relatively low cell permeability, and they act generally as irritant in moderate concentrations and caustics in high concentrations [31]. Astringents are widely used in medicines especially in dental care to cleanse, tighten gums and detoxify them and remove plaque from teeth [32].

Dr CP Boghani in his study on gingiva, had documented that biopsies taken after 15 days of use of tooth brush, Jenocin gum massage (whose main ingredient is astringent i.e. tannic acid) and G 32, revealed that the thickness of keratin layer is increased [29]. Butcher and Mitchell after conducting study on edentulous mucosa has advised the patients to frequently remove a denture & rinse the mouth with astringent to maintain activity of the palatal glands and to keep the mucous membrane in a good physiologic state [30]. However, there is no experimental evidence in the available literature demonstrating the stimulation of edentulous mucosa with astringent as a preprosthetic treatment; therefore this study was conducted to evaluate the effect of stimulation of edentulous mucosa with the use of astringent gum massage.

As per the literature, most of the studies related to effect of astringent on oral mucosa were carried out on biopsy samples [29, 30]. This study is one of its kinds as exfoliative cytological method was used in contrast to the conventional biopsy sample method. The normal turnover rate of epithelium of gingiva is about 41–57 days [33]. In our study; the keratinization process gradually fastened after the astringent massage over a period of 30 days. This increased keratinization process is beneficial for the health of edentulous mucosa under denture base.

However, the limitation of our study is limited sample size. The prolonged effect of astringent on the keratinization of denture bearing mucosa was not studied and further studies should be carried out to evaluate this effect.

Conclusion

Stimulation of the denture bearing mucosa with astringent gum massage resulted in gradual increase in keratinization. So, patients should be advised to use astringent gum massage as a preprosthetic measure to maintain the keratinization of the denture bearing mucosa.

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