

ASSESSMENT OF THE EFFECT OF COMPLETE DENTURE ADHESIVES ON ORAL EPITHELIAL CELLS' DNA IN ELDERLY PATIENTS WITH CONTROLLED DIABETES

Noha H. El-Shaheed*, Amira M. Gomaa** and Ashraf Zakaria***

ABSTRACT

Aim of the study: Denture adhesives have long been recognized by denture wearers as a valuable assistant to denture retention and stability especially for patients reporting dissatisfaction with conventional dentures as diabetic patients. There is no available guide to select the proper adhesive form with least effect on oral mucosa. This clinical study assessed the impact of different complete denture adhesive forms on oral epithelial cells' DNA in elderly patient with controlled diabetes.

Materials and Methods: Fifteen completely edentulous controlled diabetic patients were selected and complete dentures were constructed. After two weeks of complete denture insertion, patients were prescribed with denture adhesives for another two weeks. According to the used adhesive form, patients were divided into three equal groups; group (I) used denture adhesive paste, group (II) used denture adhesive powder, where group (III) used denture adhesive cushion. The maxillary alveolar mucosae of all patients were swabbed to collect epithelial cells before denture insertion, two weeks after wearing the denture and two weeks after adhesive application. DNA analysis was carried out for the swabbed epithelial cells. The percentage of Genomic Template Stability (GTS %) was assessed and compared for all patients at various time intervals.

Results: Genomic Template Stability decreased significantly in all patients two weeks after denture insertion while, significant increase in GTS% was observed two weeks after denture adhesives application. Cushion adhesive showed a significant increase in GTS% compared to paste and powder adhesives.

Conclusion: Genomic Template Stability of controlled diabetic denture wearers improved when paste, powder or cushion adhesives were used. Cushion adhesive had a better effect on GTS% compared to paste or powder adhesives.

KEY WORDS: Complete dentures, denture adhesives, diabetes mellitus, biocompatibility, epithelial cell s'DNA.

* Lecturer of Removable Prosthodontics, Faculty of Dentistry, Mansoura University, Mansoura, Egypt

** Associate Professor of Removable Prosthodontics, Faculty of Dentistry, Mansoura University, Mansoura, Egypt

*** Associate Professor of Molecular Cell Biology, Urology and Nephrology center, Mansoura University, Mansoura, Egypt

INTRODUCTION

Diabetes mellitus is one of the most dominant systemic diseases worldwide. It is commonly found in dental patients and positively correlated with edentulism⁽¹⁾.

From the perspective of oral rehabilitation, it was claimed that diabetic patients present a number of oral problems that impair the use of conventional complete denture. Because of xerostomia, the oral mucosa in diabetic patient loses the resiliency necessary for good adaptation of complete denture⁽²⁾. Factors like decreased blood supply to the tissues because of microvascular angiopathy increase the rate of residual ridge resorption⁽³⁾. Additionally, diabetes mellitus increases the susceptibility to erosion and ulceration of the mucosa where it comes in contact with the tissue surface of the complete denture⁽⁴⁾. Therefore, denture retention, stability, and comfort may be impaired⁽²⁾.

In an attempt to overcome these problems, the use of denture adhesive was demonstrated as a potential aid to improve the retention and stability of complete denture^(5, 6). **Boguck**⁽⁷⁾ has conducted a study using denture adhesives on patients affected by xerostomia and demonstrated higher stability and retention of their maxillary complete denture than without denture adhesive. Denture adhesives could be a solution for these cases and for other situations impeding the achievement of proper stability and retention of their complete dentures. By improving stability and retention, patients have increased bite force, improved efficiency of chewing, a more evenly distributed load on the basal bone decreasing the formation of mucosal ulcers⁽⁸⁾.

Concerns about the potential deleterious effects that denture adhesives may have on oral health have focused on microbial contamination and toxicity. *In vivo* studies revealed that the denture adhesive did not significantly alter the oral mucosa or microbial colonization compared to their base control in both diabetic and non-diabetic patients^(9,10,11).

On the other hand, most currently available products have been shown to be cytotoxic to the primary human oral keratinocytes, cell culture, and fibroblasts *in vitro*^(12,13,14). Many questions remained about their biologic safety *in vivo*⁽¹⁵⁾ particularly in patients with a tendency to premature DNA fragmentation in the oral masticatory epithelium under denture pressure as diabetic patients⁽⁴⁾. Molecular geneticists have provided a number of new, fast, and reliable methods for genotoxicity measurements. One of these is the Random Amplified Polymorphism DNA (RAPD) fingerprinting technology. RAPD fingerprinting of these assays was successfully applied to detect genomic DNA alterations, and difference in RAPD profiles can clearly be shown when comparing DNA fingerprints from unexposed and exposed DNA to the genotoxic agent⁽¹⁶⁾.

Hence; this study was attempted to evaluate the response of oral epithelial cells' DNA under maxillary heat cured acrylic resin denture bases to different forms of denture adhesives in diabetic patients compared to its response to maxillary heat cured acrylic resin denture bases without denture adhesives.

MATERIALS AND METHODS

Subject selection and protocol

This study was conducted after approval from the Ethics and Research Committee of Faculty of Dentistry, Mansoura University.

Fifteen completely edentulous controlled glycaemia patients with age ranging from 50-65 years were selected. For the selected patients, glycemic control was ensured by evaluating the glycated hemoglobin (HbA1c) level. Recommendation for strict glycemic control for persons with diabetes have targeted maximal HbA1c levels ranging from 6.5% up to 7.0%⁽¹⁷⁾. Patients with powerful confounders known to affect DNA as those with

history of cancer; previous radio- or chemotherapy; smokers, and patients exposed to diagnostic X-rays during the last 6 months were excluded^(18,19).

For every patient, maxillary and mandibular complete dentures were constructed following a standardized protocol. The processed dentures were laboratory and clinically remounted for occlusal adjustments. Final occlusal adjustment was ensured intra orally. All the patients were instructed to clean their dentures using water and brush only during the period of this study. Patients were recalled after two weeks for follow up evaluation and sample collection.

Patients were prescribed with denture adhesives (Protefix[®] brand, Queisser Pharma 24914 Flensburg-Germany) for another two weeks. According to the denture adhesive forms patients were randomly divided into three equal groups as follows; group (I) included patients prescribed with denture adhesive paste,; groups (II) included patients prescribed with denture adhesive powder; groups (III) included patients prescribed with denture adhesive cushions. Patient's instructions and a demonstration were performed about the use of the adhesive according to the manufacturers' recommendations.

Two weeks after using denture adhesive, patients were recalled for a second follow up evaluation and sample collection.

Sample collection and DNA extraction

The maxillary alveolar mucosae of all patients were swabbed to collect epithelial cells before denture insertion for baseline analysis, two weeks after wearing the denture and two weeks after adhesive application. Before sample collection, patients were allowed to rinse with anti-septic mouth wash, followed by sterile saline. Epithelial cells were collected by scraping the examination areas

most likely to come into contact with the maxillary denture with sterile blunted metal spatula. These areas were: the crest of the maxillary ridge and buccal and palatal to the crest of the ridge in the first molar area⁽²⁰⁾ (**Fig.1**). Cells were then transferred to a tube containing 0.5 ml saline solution, centrifuged (13 000 rpm) for 3 minutes, and the DNA was extracted using DNA extraction kit (Wizard Genomic DNA purification kit, Promega, USA). The DNA was rehydrated in 10-50 ml of DNA rehydration solution for 1 hour at 65° C or overnight at 4° C.



Fig. (1) Sample collection.

Agarose gel electrophoresis of the extracted DNA

2 μ l of each DNA sample was mixed with 2 μ l blue/orange 6x loading dye and resolved by electrophoresis on 1.5% agarose gel at 100 V (Volts) for 3 h in 1X Tris Acetate EDTA (SERVA Electrophoresis GmbH) mixed with ethidium bromide (Sigma, USA) (5 μ l/100 ml agarose) and 4 μ l DNA marker II (Fermentas, EU) "702-29946 bp" mixed with the same loading dye was loaded into the first well in the same gel.

DNA integrity and mobility rate were examined under Ultra Violet light (Cole-Parmer, France) and instantly photographed (**Fig.2**).

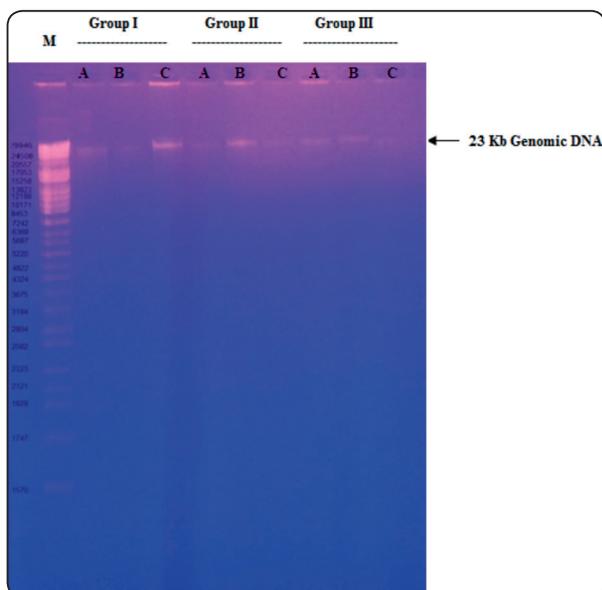


Fig. (2) Agarose gel electrophoresis of extracted genomic DNA. M: DNA marker II "702-29946 bp".

DNA fingerprint analysis by Random Amplified Polymorphic DNA Polymerase Chain Reaction {RAPD PCR}⁽²¹⁾

1 μ g of pure genomic DNA and 15 pm of the selected primer (Operon kit A-2, USA), with the sequence 5'-TGCCGAGCTG-3' were mixed with one PCR bead (PCR-GOLD Master-Mix beads, Biron, Germany) in 200 μ l sterile eppendorf and the final volume was completed to 20 μ l with sterile distilled water. PCR reaction mixture was then transferred to thermal cycler (Techne, UK) programmed for 40 cycles in 1 minute at 94°C for DNA denaturing, then 1 minute at 40°C for primer annealing with DNA and 2 minutes at 72 °C for DNA extension. A second program as one cycle for 7 minutes at 72°C for final DNA extension was performed. Finally; the reaction was terminated by holding the tubes at 4°C in the PCR machine as a third program.

RAPD PCR electrophoresis

10 μ l of the amplified product was mixed with 2 μ l blue/orange 6x loading dye and resolved by electrophoresis on 1.5% agarose gel at 100 V (Volts) for 3 h in 1X Tris Acetate EDTA (SERVA

Electrophoresis GmbH) mixed with ethidium bromide (Sigma, USA) (5 μ l/100 ml agarose) and 4 μ l 1 kb ladder DNA (250-10,000 base pairs, AXYGEM, USA) was used as a marker. The bands were visualized and documented using the UV gel documentation system (Cole-Parmer, France) (Fig. 3).

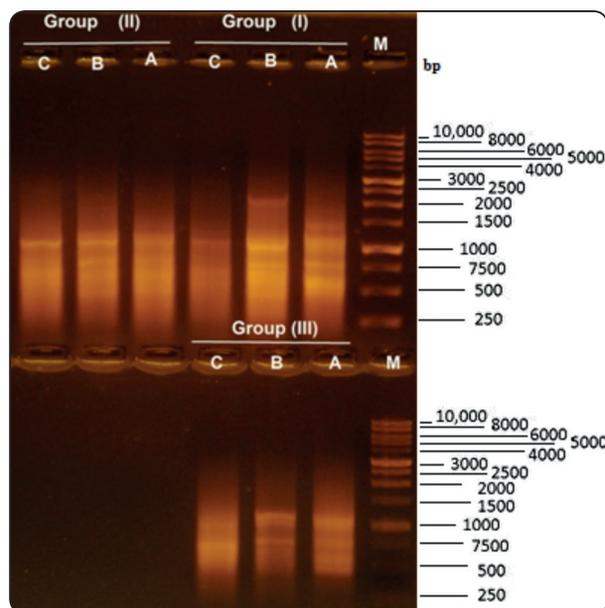


Fig. (3) RAPD-PCR profile of DNA samples of three different patients in group (I), (II), (III) before denture insertion (A), two weeks after denture insertion (B), and two weeks after denture adhesive application (C). M: 1 kb DNA marker.

RAPD-PCR analysis

- Qualitative analysis**⁽²²⁾ Polymorphism in RAPD profiles, including disappearance of a normal band and appearance of a new band of particular molecular weight in comparison to the control RAPD profile, was noted.
- Quantitative analysis**⁽²³⁾: The bands in PCR products were analyzed. The resulted bands were marked on the gel photograph, and their molecular weights were calculated using software image analysis (GelAnalyzer, 2010) (Fig.4). Each change observed in the DNA molecular weights compared with control

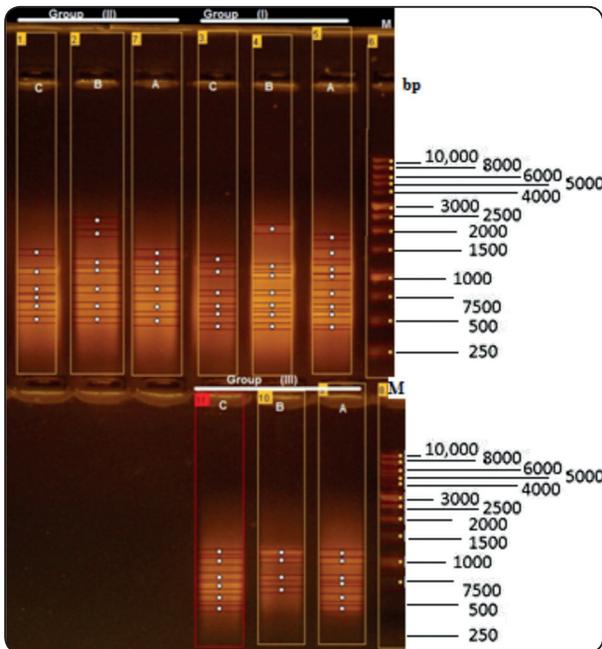


Fig. (4) RAPD-PCR profile of the DNA samples of groups I, II and III. All bands were marked on the gel using GelAnalyzer software. A: before denture insertion ,B: two weeks after denture insertion, and C:two weeks after denture adhesive application. M 1 kb DNA marker.

(before denture insertion) was scored as +1. The percentage of Genomic Template Stability (GTS%) two weeks after wearing the denture, and two weeks after adhesive application in comparison to their controls (set as 100%) has been calculated as: $GTS \% = 100 - (100 a/n)$, where 'a' is the average number of changes in DNA molecular weights and 'n' the number of bands in the control DNA.

Statistical analysis

The analysis was carried out with a statistical system, SAS, 8-1-2004).

Mean and standard deviation was used to describe data. Repeated measure ANOVA was used to test for significant difference of GTS% within the same group at various time intervals. Paired t-test was used to test for significant difference between each two time interval within the same group.

One way ANOVA test was used to test for significant difference of GTS% between different groups at each time interval. Independent t -test was used to test for significant difference between each two groups at each time interval. Differences were considered significant with a probability level of $P < 0.05$.

RESULTS

RAPD profiles revealed significant differences between before and two weeks after denture insertion with obvious changes in the number and size of amplified DNA bands in all groups. Most of the observed changes after two weeks were temporary and was repaired two weeks after denture adhesive application and the few were persistent (**Fig.3**).

On the light of statistical analysis for each group at various time intervals, it has been noted that, for all groups, there was a significant change in GTS % between the three times interval (A,B,C) (Repeated measure ANOVA test $P < 0.0001$). The results of t-test showed that, the Genomic Template Stability (GTS%) decreased significantly in all groups (paste (I), powder(II) and cushion(III)) two weeks after denture insertion (B) compared to control (A). Two weeks after denture adhesives application (C), significant increase in (GTS %) of all groups was evident compared to GTS % of two weeks after denture insertion (B) (**Tab. 1**).

Means with the same letter are not significantly different.

On the light of statistical analysis between the three different groups at each time intervals, it has been noted that, at two weeks after denture insertion (B), there was non-significant difference between the three groups (one way ANOVA $p = 0.14$). At two weeks after denture adhesive application (C), there was a significant difference of GTS% between the three groups (one way ANOVA $p < 0.0001$). The results of t-test showed that, the (GTS%) increased significantly in group (III) (cushion) compared to groups (I) and (II) (paste and powder).(**Tab.2**)

TABLE (1) Descriptive statistics and significant change in the GTS% for each group at various time intervals using repeated measure ANOVA test:

| Time \ Group | Before denture insertion (A) Mean \pm SD | Two weeks of denture insertion (B) Mean \pm SD | Two weeks of adhesive application (C) Mean \pm SD | F value | P value |
|----------------------|---|---|--|---------|---------|
| Group (I) (paste) | 100.00 \pm 0.00a | 45.00 \pm 6.52c | 62.5 \pm 1.22b | 314.30 | <0.0001 |
| Group (II) (powder) | 100.00 \pm 0.00a | 50.00 \pm 1.00c | 72.50 \pm 1.90b | 1538.11 | <0.0001 |
| Group(III) (cushion) | 100.00 \pm 0.00a | 50.00 \pm 1.22c | 83.50 \pm 2.06b | 1693.26 | <0.0001 |

S.D.: Standard deviation.

P < 0.05 significant.

TABLE (2) Descriptive statistics and significant change in the Genomic Template Stability GTS% between the three different groups at each time interval using one way ANOVA:

| Time \ Group | Group (I) (paste) Mean \pm S.D. (n=10) | Group (II) (powder) Mean \pm S.D. (n=10) | Group(III) (cushion) Mean \pm S.D. (n=10) | F value | P value |
|--|--|--|---|---------|---------|
| Before denture insertion (A) | 100.0a \pm 0.0 | 100.0a \pm 0.0 | 100.0a \pm 0.0 | - | - |
| Two weeks after denture insertion (B) | 45.00 \pm 6.52a | 50.00 \pm 1.0a | 50.00 \pm 1.22a | 11.91 | 0.14 |
| Two weeks after denture adhesive application (C) | 62.5 \pm 1.22c | 72.50 \pm 1.90b | 83.50 \pm 2.06a | 466.53 | <0.0001 |

S.D.: Standard deviation.

P < 0.05 significant.

Means with the same letter are not significantly different.

DISCUSSION

In situations where denture foundation is not favorable or in cases where salivary flow is compromised due to systemic diseases such as in diabetes mellitus, the denture adhesive is recommended as an aid to enhance retention and stability of complete dentures⁽⁷⁾.

The biological safety of denture adhesives in clinical application should be explored in these patients as they are frequently prescribed to improve their prosthetic performance. So it has become imperative to evaluate the tissue response to these materials and which material form suits best. This

was the aim of this study which was conducted on the use of conventional heat cured acrylic resin followed by the use of different forms of denture adhesives including paste, powder, and cushion to explore and compare their effect on oral epithelial cells' DNA of controlled diabetic patients.

A serious concern is the potential for exposure to genotoxic agents because the genotoxic effect of a material can induce changes in the genome that disrupt its integrity or function. Depending on the intensity of that effect, the cell could recover, start neoplastic growth, or die⁽²⁴⁾. So, in this study, a specific attempt was made to test the effect of these materials on oral epithelial cells' DNA.

The epithelial cell kinetics is especially important in the interpretation of the results. DNA damage occurs in the basal layer of epithelium, where the cells undergo mitosis, but it is observed later in exfoliated cells after differentiation. The turnover of this epithelium is rapid (from 7 to 16 days), and thus the maximal rate of DNA damage is expected after about two weeks subsequent to the exposure to a genotoxic agent⁽²⁵⁾. For this reason, the first evaluation was done two weeks after denture insertion. The second evaluation was performed after another two weeks of denture adhesive application to assess DNA repair kinetics and/or to evaluate the persistence of the induced DNA effects.

Genetic alterations were detected in the oral epithelial cells' DNA two weeks after denture insertion as polymorphic amplification fragments caused by gain or loss of particular fragment reflected in their molecular weight change. This is explained by the primer anneals evident during the PCR process resulting in complementary sequences of the DNA before the insertion of the denture; and if after the insertion the mucosa DNA is changed, so the annealing sites of the primer would be changed; and so the original DNA bands disappear or another DNA bands appear⁽²¹⁾.

This was expressed quantitatively as significant decrease in the Genomic Template Stability GTS% two weeks after denture insertion compared to GTS% before denture insertion. This probably indicates that heat-cured acrylic resin was able to produce genetic alterations in the denture supporting oral mucosa of diabetic patients two weeks after wearing maxillary dentures.

The molecular mechanism responsible for the observed genetic alterations could be due to either direct genetic damage or an oxidative stress created by resin monomers which could interfere with the DNA molecules and create changes in their sequence. This ultimately results in the formation of new priming sites and/or disappearances of existing priming sites for the RAPD primers and thus

gives inconsistent RAPD profiles after denture insertion⁽²⁶⁾.

Clinically, the unreacted monomers may be released from the resin materials into the aqueous environment of oral cavity even after complete polymerization of the resin⁽²⁷⁾. The concentration level of methyl methacrylate (9.3310 mg/ml) could easily reach the effective cytotoxic and genotoxic level. It is possible that the amount of methyl methacrylate released into oral mucosa may be higher⁽²⁸⁾ and could easily reach the effective genotoxic level, especially in the area under the denture^(29,30). The loss of the normal tissue protective barrier in diabetic patients due to progressive atrophy of oral mucosa may increase the permeability of the oral mucosa to resin residual monomer and probably leading to more DNA damage^(4,31).

Significant increase in (GTS%) that was evident two weeks after paste, powder and cushion forms of denture adhesive application in diabetic denture wearers could denote some DNA repair and improvement in the biologic condition of the oral mucosa. This finding may be attributed to formation of a barrier between denture and mucoperiosteum as a result of adsorption of water and saliva by adhesives resulting in formation of an anionic layer, that is attracted to cationic protein present in the mucous membrane this in turn may produce stickiness and form a barrier between denture and mucoperiosteum as previously reported⁽³²⁾. Additionally, this could be attributed to the enhanced retention and stability caused by the use of denture adhesives which may improve the biologic condition of the oral mucosa including reduction of mucosal irritation, reduction of food impaction beneath the denture base, improvement of chewing efficiency, and improvement of functional load distribution across the denture-bearing tissues as reported by previous studies^(7,8,9).

On the other hand, denture adhesives have been evaluated *in vitro* and showed different degrees of cytotoxicity^(12,13,14). While another study reported that Protefix® cream and powder

which was used also in this study, did not cause evident cytotoxicity⁽³³⁾. Another study showed that Protefix® powder showed low cytotoxicity, whereas Protefix® cream was non-cytotoxic⁽³⁴⁾. However, the clinical implication of such in vitro findings is difficult to ascertain as the oral mucosa is more resistant to toxic ingredients than a cell culture, because of the mucin and the keratin layers. In addition, the dynamic environment of the oral cavity may influence the cytotoxicity parameters, such as fluctuations of salivary flow, temperature and pH, together with variable muscle movements. Therefore, the cytotoxicity of denture adhesives in vivo is liable to be lower than that in monolayer cell cultures⁽³⁴⁾.

In this study, the cushion form showed significant improvement compared to other forms. This improvement may be because a cushion adhesive acting as a relining material can adjust to both the supporting tissues and the intaglio of the denture. It provides a cushioning effect, reducing the pressure and friction transmitted to the underlying mucosa⁽³⁷⁾. In addition, it prevents direct contact with surfaces, possibly reducing the genotoxic effect of acrylic resin denture base material. Also, the action of the cushion adhesive has been reported to remain after 10 hours, while the majority of paste adhesives remained active for over 4 hours but fewer than 10 hours^(36,38). This prolonged effect may provide farther protection against genotoxic agents.

This result was in agreement with previous subjective studies that reported a cushion adhesive to be the most effective in clinical improvement in patients with poor/ fair dentures or prosthesis-bearing tissues^(35,36).

This study showed that the powder form of denture adhesive revealed significant improvement compared to paste form. These results may have been attributed to the increased viscosity of the paste denture adhesives as compared to the powder form⁽³⁹⁾ and this results in a slight increase in microbial colonization for the paste form of denture

adhesive as compared to the powder form as reported by a previous study⁽⁴⁰⁾. This microbial biofilm may be responsible for the lower degree of oral mucosal improvement of paste form in this study.

Molecular biological data obtained in the present study afford valuable visions into the genetic effects of different forms of denture adhesives on the oral epithelial cells' DNA in diabetic patients. Generally, normal cells can repair these lesions. Loss of repair capability might be a starting occurrence of adverse biologic effects. Nature and amount of DNA effects can only be categorized if alterations arising in RAPD profiles are analyzed by sequencing or probing.

LIMITATIONS OF THIS STUDY

Some limitations of this study were the absence of multiple brands of denture adhesives, small study population and relatively short trial period.

CONCLUSION

Within the limitation of this study, it can be concluded that:-

1. The marketable denture adhesives used in this study clarified marked improvement in the Genomic Template Stability (GTS%) of all groups two weeks of adhesives application.
2. The use of cushion adhesive form exhibited significant increase in GTS% compared to other forms in diabetic patients.
3. When complete denture adhesive is advised to enhance denture retention for diabetic patient, cushion form is recommended.

RECOMMENDATION

Further studies must include an extended period of assessment, as the patients who use adhesives use the product for extended periods of time, as well as compare different products in order to determine the actual effect of the adhesives on oral mucosa.

REFERENCES

1. Felton DA. Edentulism and comorbid factors. *J Prosthodont.* 2009; 18:88-96.
2. Madhup A, Godbole SR, Meshram S. Comparative evaluation of stimulated salivary flow in between diabetic and non-diabetic subjects wearing complete denture. *International J. of Healthcare and Biomedical Research*, 2015; 04: 40-43.
3. Al-Jabrah O. Association of type 2 diabetes mellitus with the reduction of mandibular residual ridge among edentulous patients using panoramic radiographs. *Open J. of Stomatology.* 2011; 1: 61-68.
4. Maruo Y, Sugimoto T, Oka M, Sato T. Accelerated DNA fragmentation of the denture-bearing mucosal epithelium in an animal model of diabetes. *J. of Oral Rehab* 2001; 28:393-9.
5. Yegin E, Akpınar YZ, Yavuz T & Aslan MA: Effect of different denture adhesives on retention of complete dentures: an in vivo study *J. of Adhesion Science and Technology.* 2017; 31: 2044-2052.
6. El-Mekawy N, Gomaa AM, and Habib AA: Comparison of three different forms of denture adhesives: Direct measurement of denture retention and, study of patient satisfaction *Egypt Dent J.* 2012; 58:1-9.
7. Bogucki ZA. Denture adhesives' effect on retention of prostheses in patients with xerostomia. *Adv Clin Exp Med.* 2018;27:1-6.
8. Gonçalves TM, Viu FC, Gonçalves LM, Garcia RC. Denture adhesives improve mastication in denture wearer. *Int J Prosthodont.* 2014;27:140-6.
9. Jadhav R, Jadhav R, Rayannavar S, Patil R, Hiremath V, Hogade SA. Effect of denture adhesive and its forms on microbial colonization in diabetic and non diabetic complete denture wearers: An in vivo study. *Indian J Health Sci Biomed Res.* 2017; 10:131-137.
10. Borole A, Roopa K T, Khandelwal PV. "A comparative evaluation of the effects of different commercially available denture adhesives on the growth of *Candida* species in diabetic and nondiabetic subjects:" An In vivo Study. *J Dent Allied Sci* 2016; 5:63-9.
11. Oliveira MC, Oliveira VM, Vieira AC, Rambob I. In vivo assessment of the effect of an adhesive for complete dentures on colonisation of *Candida* species. *Gerodontology.* 2010;27:303.
12. Chen F, Wu T, Cheng X. Cytotoxic effects of denture adhesives on primary human oral keratinocytes, fibroblasts and permanent L929 cell lines. *Gerodontology* 2014; 31: 4-10
13. Al RH, Dahl JE, Morisbak E, et al: Irritation and cytotoxic potential of denture adhesives. *Gerodontology* 2005;22:177-183.
14. De Gomes PS, Figueiral MH, Fernandes MH, Scully C. Cytotoxicity of denture adhesives. *Clin Oral Invest.* 2010; 15:885-893. 16.
15. Papadiochou S, Emmanouil I, Papadiochos I. Denture adhesives: a systematic review. *J Prosthet Dent.* 2015; 113:391-397.
16. Cenkci S., Cigerci I. H., Yildiz M., Ozay C., Bozdog A., Terzi H.: Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environ Exp Bot* 2010; 67: 467-473.
17. Standards of medical care in diabetes. *Diabetes Care.* 2010; 33(Suppl 1):511-61.
18. Minicucci E.M., Kowalski L.P., Maia M.A., Pereira A., Ribeiro L.R., de Camargo J.L., Salvadori D.M.: Cytogenetic damage in circulating lymphocytes and buccal mucosa cells of head-and-neck cancer patients undergoing radiotherapy. *J Radiat Res* 2005; 46:135-142.
19. Sinko I., Morocz M., Zadori J., Kokavszky K., Rasko I.: Effect of cigarette smoking on DNA damage of human cumulus cells analyzed by comet assay. *Reproductive Toxicology* 2005; 20: 65-71.
20. Pickett H.G., Appleby R.C.: A comparison of six acrylic resin processing techniques. *J Amer Dent Assos.* 1970; 80: 1309-1314.
21. Williams J., Kubelik A. R., Livak K. J.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* 1990; 18: 6531-6535.
22. Fritsch P., Rieseberg L.H.: The use of random amplified polymorphic DNA (RAPD) in conservation genetics. In: T.B. Smither, R.K. Wayne (Eds.), *Molecular Genetic Approaches in Conservation*, Oxford University Press, (1996); pp. 55-73.
23. Atienzar F.A., Cordi B., Donkin M.E., Evenden A.J., Jha A.N., Depledge M.H.: Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmate*. *Aquatic Toxicology.* 2000.; 50: 1-12.

24. Swift LH, Golsteyn RM. Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells. *International J. Molecular Sciences* 2014;15:3403–3431.
25. Dawes C.: Estimates, from salivary analyses, of the turnover time of the oral mucosal epithelium in humans and the number of bacteria in an edentulous mouth. *Archives of Oral Biology*. 2003; 48: 329-336.
26. Att W., Yamada M., Kojima N., Ogawa T.: N-acetyl cystiene prevents suppression of oral fibroblast function on polymethyl methacrylate resin. *Acta Biomater* 2009; 5:391- 398.
27. Schweikl H., Hartmann A., Hiller K. A., Spagnuolo G., Bolay C., Brockhoff G.: Inhibition of TEGDMA and HEMA-induced genotoxicity and cell cycle arrest by N-acetyl cysteine. *Dent Mater*. 2007; 23:688–695.
28. Baker S., Brooks S. C., Walker D. M.: The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: an assay for monomer in saliva. *J Dent Res*.1988; 67: 1295-1299.
29. Yang H., Chou L., Chou M., Chang Y.: Assessment of genetic damage by methyl methacrylate employing in vitro mammalian test system. *Biomaterials*. 2003; 24: 2909-2914.
30. El Shaheed N, El Khodary M, Gomaa A, Zakaria A. Impact of reinforced heat cured acrylic resin on oral epithelial cells (DNA) analysis). *Egypt Dent J* .2011; 57:941-950.
31. Ujpal M, Matos O, Bibok G, Somogyi A, Szabo G, Suba Z. Diabetes and oral tumors in Hungary: epidemiological correlations. *Diabetes Care* 2004; 27:770–4.
32. Kumar PR, Shajahan PA, Mathew J, Koruthu A, Aravind P, Ahammed MF. Denture Adhesives in Prosthodontics: An Overview. *J Int Oral Health*. 2015; 7(Suppl 1): 93–95.
33. Al RH, Dahl JE, Morisbak E et al. Irritation and cytotoxic potential of denture adhesives. *Gerodontology* 2005; 22: 177–183.
34. de Gomes PS, Figueiral MH, Fernandes MH et al. Cytotoxicity of denture adhesives. *Clin Oral Investig* 2011; 15: 885–893.
35. Uysal H, Altay OT, Alparslan N, Bilge A. Comparison of four different denture cushion adhesives-a subjective study. *J Oral Rehabil* 1998;25:209-13.
36. Koronis S, Pizatos E, Polyzois G, Lagouvardos P. Clinical evaluation of three denture cushion adhesives by complete denture wearers. *Gerodontology* 2012;29:e161-9.
37. Abdelmalek RG, Michael CG. The effect of denture adhesives on the palatal mucosa under complete dentures. A clinical and histological investigation. *Egypt Dent J* 1978;24:419-30.
38. Kulak Y, Ozcan M, Arikan A. Subjective assessment by patients of the efficiency of two denture adhesive pastes. *J Prosthodont* 2005;14:248-52.
39. Chowdhry P, Phukela SS, Patil R, Yadav H. A study to evaluate the retentive ability of different denture adhesive materials: An in vitro study. *J Indian Prosthodont Soc* 2010;10:176-81.
40. Makihira S, Nikawa H, Satonobu SV, Jin C, Hamada T. Growth of *Candida* species on commercial denture adhesives in vitro. *Int J Prosthodont* 2001;14:4852. 5.