AMNIOTIC MEMBRANE VERSUS SUBEPITHELIAL CONNECTIVE TISSUE GRAFT IN THE MANAGEMENT OF THIN GINGIVAL BIOTYPE USING VESTIBULAR INCISION SUBPERIOSTEAL TUNNEL ACCESS TECHNIQUE (A RANDOMIZED CLINICAL TRIAL)

Ahmed Abdallah Khalil

ABSTRACT

Background: Gingival biotype used to describe the thickness of the gingiva in the facio-palatal dimension, and it mainly refers to the quality of the soft tissue profile surrounding the teeth. The current study was designed to compare the effect of amniotic membrane and Subepithelial connective tissue graft in the management of thin gingival biotype.

Subjects and methods: 20 Subjects with thin gingival biotype in lower anterior area randomly assigned into two equal groups using Vestibular Incision subperiosteal Tunnel Access (VISTA) technique, Group (A) including ten subjects with Subepithelial connective tissue graft (SCTG) was inserted through subperiosteal tunnel to cover the gingiva of lower incisors area. Group (B) including ten subjects with cryopreserved amniotic membrane (AM) was inserted through subperiosteal tunnel covered the gingiva of lower incisors area. Following parameters were evaluated before treatment (baseline), after three and six months post operative: Plaque index (PI), gingival index (BI), Probing depth (PD), Width of keratinized tissue (WKT) and thickness of keratinized tissue (TKT).

Results: Both group A and group B showed significant improvement regarding all clinical parameters, from baseline to six months except in WKT. There was significant improvement regarding TKT in SCTG group compared to AM group at 6 months interval.

Conclusion: Both SCTG and AM show clinical improvement in management of thin gingival biotype. There was a significant improvement regarding TKT in SCTG group compared to AM group at 6 months interval.

KEYWORDS: Thin biotype, Amniotic membrane, Subepithelial connective tissue graft, VISTA technique.

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INTRODUCTION

Thin gingival biotype is a common risk factor for gingival and periodontal diseases as severe alveolar bone resorption in the apical and lingual directions are more possibly to occur during the remodeling process after tooth extraction. Thin gingival biotypes are fine, greatly scalloped, and translucent in appearance. The tissue appears delicate, friable with a small band of the attached gingiva. The underlying bone; is thin and fenestrations or dehiscence may be present.

Moreover, much of responsibility has also been placed on the soft tissue biotype and its effect either as an etiological or modifying factor leading to recession.

Treatment modalities for thin gingival biotypes include subepithelial connective tissue graft (SCTG) with different variations demonstrated with highest success rates and greatest amount of predictability. This technique was firstly described by Langer and Langer in 1985 and has had numerous variations in the surgical technique described since.

SCTG has been broadly used for increasing the width and thickness of keratinized gingiva around natural teeth or dental implants to withstand forces and prevent or manage recession. Nevertheless, an extra surgical site for harvesting the graft is accompanied with pain, morbidity, and maybe imperfected quality or little amount of the tissue restrict their use. To avoid these difficulties, different alternatives have been developed for soft tissue augmentation.

Amniotic membrane (AM) or amnion, which is the most internal layer of human placenta, consists of thick layer of basement membrane, and an avascular stromal matrix. It has been used for a long time with high success rate in different types of surgeries. Several properties of AM permit its use in the management of burns and ocular surgery. These include encouragement of epithelialization, Anti-scarring effect, Anti-inflammatory effect, Presence of growth factor, Expression of stem cell markers.

In addition, AM has many applications in periodontics. It has a good result in increasing the thickness of the soft tissue, management of gingival recession and increasing the attached gingiva with excellent outcomes in terms of texture and color matching. Also, it act as a barrier in cases of intra bony defects and furcation involvement.

In vestibular incision subperiosteal tunnel access technique (VISTA), the incision is made in the vestibule where an access to the underlying alveolar bone, root dehiscence and the entire region can be achieved by single incision. this vestibular incision reduces the trauma of the soft tissue around the teeth. To reduce the tension of the gingival margin during coronal movement of the tissue; a careful subperiosteal dissection is must. Also, VISTA technique can maintain the integrity of interdental papillae while by avoiding its reflection.

Hence, The present study was carried out to compare subepithelial connective tissue graft and amniotic membrane clinically in management of thin gingival biotype using VISTA technique.

SUBJECTS AND METHODS

This study was conducted on total of 20 subjects with thin gingival biotype in the lower anterior area that were randomly divided by using toss coin into two equal groups Group (1) using SCTG and Group (2) using AM. The subjects were selected from the outpatient clinic of the oral medicine, Oral diagnosis, and Periodontology Department, Faculty of Dentistry- Minia University.

Sample size calculation:

A power analysis was designed to have appropriate power to apply a statistical test of the null hypothesis that there is no difference would
be found in clinical parameters between the two groups. By adopting an alpha level of (0.05) a beta of (0.2) i.e. power=85% and an effect size (d) of (1.5117587), the predicted sample size (n) was a total of (9) samples and 10% from total sample size was added to adjust for follow up loss. So, the final total sample size was 10 for each group with total 20 cases for the whole study. Sample size calculation was performed using G*Power version 3.1.9.7 (15).

**Ethical regulations:**

The complete treatment plan including detailed steps, risks, and expected results was explained to all participants and their full signed consent was obtained before entry into the research. The study complied with the rules of the International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki. The research was agreed by the research ethics committee of the Faculty of Dentistry- Minia University No. (674) on twenty-seven of December 2022.

**Subject selection:**

Age of selected subjects of both sexes ranged from 25-48 years old. Subjects exhibited signs of thin gingival biotype (Thickness less than 1.5 mm according to Claffey and Shanley definition) (16). The subjects were free from any systemic diseases according to questionnaire dental modification of Cornell index and had not undergone any type of periodontal treatment six months before the first examination. on the other hand, cases with recession, pregnant, lactating females, and smokers were excluded from the research.

**Treatment steps:**

- All subjects underwent phase I therapy including full mouth mechanical debridement, supra and sub-gingival scaling and root planing using Chlorhexidine mouthwash HCL 0.12% (Hexitol mouth wash -the Arab Drug company-Cairo-Egypt), twice daily for 14 days. All subjects were reevaluated two weeks after first treatment, and the subjects were randomly divided into two equal groups each one have 10 subjects Group (1) using Subepithelial connective tissue graft. Group (2) using cryopreserved amniotic membrane.
- For standardization of assessments, preoperative impression was taken for all subjects for making a vacuum stent with holes represent the specific area where the measurement of thickness and width of keratinized tissue were taken Fig (1).
- The operative area was anesthetized by using 2% lignocaine HCl with adrenaline (1:2,00,000) using infiltration techniques in lower anterior area.
- The VISTA technique using VISTA TUNNELING KIT (ARTMAN instruments, USA) was started with a small access incision through the periosteum in the vestibule mesial to canine area at side which need to be augmented. the tunnel was made subperiosteally by inserting the different shape periosteal elevators of the kit between bone and the periosteum through the incision which provided access to the entire area Fig (2).
- For Group (1), SCTG was obtained by incision made between the distal aspect of the first molar and the distal aspect of the first premolar and with single incision technique as given by Hürzeler and Weng in 1999 (17). A SCTG with even thickness was harvested, shaped for grafting while the donor site was sutured using non resorbable 4 - 0 black silk suture. SCTG was inserted through subperiosteal tunnel to augment the lower anterior area and sutured from both terminal ends using 6-0 SUTURE - Vicryl (PGA) – Egysorb, Egypt Fig 3 (A, B & C).
• For Group (2), cryopreserved amniotic membrane (Cell tissue bank, El-kasr-Aliniy, Cairo university) was removed from the carrier (nitro cellulose paper) with epithelial side up and washed with saline before application. Then it was inserted through subperiosteal tunnel carefully to cover and augment the lower incisors area and sutured from both terminal ends using 6-0 suture- (Vicryl (PGA)-Egysorb, Egypt) Fig 4 (A & B).

Clinical evaluation:

regular assessment visits were done for all subjects at baseline (before surgery), three and six months post operative. The clinical measurements include plaque index (PI), gingival index (GI), Probing depth (PD), Width of keratinized tissue (WKT), and thickness of keratinized tissue (TKT).

![Fig. (1) using Vacuum stent for measurement of TKT.](image1)

Fig. (1) using Vacuum stent for measurement of TKT.

![Fig. (2) VISTA approach using VISTA TUNNELING KIT](image2)

Fig. (2) VISTA approach using VISTA TUNNELING KIT

![Statistical Analysis:](image3)

Statistical Analysis:

All clinical readings were recorded for statistical analysis. The mean and standard deviations were calculated for the required assessment durations. For the intragroup comparisons paired t-test was used while for intergroup comparisons independent t-test was used.

RESULTS

Both group A and group B showed significant improvement regarding all clinical parameters, from baseline to six months except in WKT, there was no statistically significant improvement in
both groups from base line to 6 months after surgery. Comparison of all clinical parameters for both groups from base line to 6 months including the mean and standard deviation is represented in Table (1). Upon comparing the results of SCTG group with AM group, no statistical significance changes were detected in all parameters except TKT. A significant improvement regarding TKT in SCTG group (1.78±.13) was found compared to AM group (1.45±.28) at six months interval (Fig.5).

**TABLE (1) Shows Comparison of all clinical parameters for both groups from base line to 6 months.**

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>Group (1)</th>
<th>Group (2)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 0</td>
<td>1.4±0.5</td>
<td>1.5±0.4</td>
<td>0.656</td>
</tr>
<tr>
<td>PI 3</td>
<td>0.8±0.4</td>
<td>0.9±0.3</td>
<td>0.463</td>
</tr>
<tr>
<td>PI 6</td>
<td>0.6±0.3</td>
<td>0.7±0.2</td>
<td>0.565</td>
</tr>
<tr>
<td>Gingival index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI 0</td>
<td>1.35±0.47</td>
<td>1.39±0.49</td>
<td>0.852</td>
</tr>
<tr>
<td>GI 3</td>
<td>0.73±0.22</td>
<td>0.79±0.26</td>
<td>0.596</td>
</tr>
<tr>
<td>GI 6</td>
<td>0.33±0.11</td>
<td>0.39±0.13</td>
<td>0.304</td>
</tr>
<tr>
<td>Probing depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 0</td>
<td>1.75±0.46</td>
<td>1.75±0.53</td>
<td>1.000</td>
</tr>
<tr>
<td>PD 3</td>
<td>1.39±0.29</td>
<td>1.27±0.34</td>
<td>0.416</td>
</tr>
<tr>
<td>PD 6</td>
<td>1.10±0.33</td>
<td>1.0±0.39</td>
<td>0.548</td>
</tr>
<tr>
<td>Thickness of KT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TKT 0</td>
<td>1.22±0.19</td>
<td>1.32±0.26</td>
<td>0.344</td>
</tr>
<tr>
<td>TKT 3</td>
<td>1.56±0.09</td>
<td>1.40±0.25</td>
<td>0.083</td>
</tr>
<tr>
<td>TKT 6</td>
<td>1.78±0.13</td>
<td>1.45±0.28</td>
<td>0.004*</td>
</tr>
<tr>
<td>Width of KT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKT 0</td>
<td>4.31±0.67</td>
<td>4.41±0.51</td>
<td>0.714</td>
</tr>
<tr>
<td>WKT 3</td>
<td>4.32±0.66</td>
<td>4.41±0.50</td>
<td>0.709</td>
</tr>
<tr>
<td>WKT 6</td>
<td>4.35±0.66</td>
<td>4.44±0.52</td>
<td>0.765</td>
</tr>
</tbody>
</table>

*P-value <0.001* (significant)
DISCUSSION

The final esthetic results affected by the gingival biotype. So, it is important for the operator to recognize the gingival biotype and to change the thin to a thick biotype (18).

Gingival thickness is a convincing factor of the results of periodontal surgeries. According to different studies, thick biotype has a better results after periodontal therapy. It is possibly related to increased blood supply, considerable perfusion with oxygen, loss of toxic products, immune response, and growth factor migration. So, thick gingival biotype is necessary for enhancing the results of surgical therapy (19). Thick tissue biotype composed of flat soft tissue and thick dense bony architecture and with great amount of attachment, which make the tissue more resistant to gingival recession, on the other hand, recession, bleeding, and inflammation are common in thin tissue biotype which is delicate with highly soft scalloped tissue and thin bony architecture (20).

The present study was carried out to compare subepithelial connective tissue graft and amniotic membrane clinically in management of thin gingival biotype using VISTA technique. The study comprised two groups: Group (1) In which 10 subjects with thin gingival biotype in the lower anterior area were augmented using Subepithelial connective tissue graft. Group (2) In which 10 subjects with thin gingival biotype in the lower anterior area were augmented using cryopreserved amniotic membrane.

The target of using VISTA technique; is providing adequate blood supply and also it is consider as a minimally invasive technique (21).

Although both groups showed an significant improvement regarding most of clinical parameters from baseline to six months, the present study did not reveal any significant differences regarding Width of keratinized tissue between the two treatment groups, or in the same group from base line to 6 months. This may be related to the technique used as no coronally advancement for the flap were done or the number of the sample size. Also, VISTA technique dose not result in increase of the keratinized gingiva, or the gingival thickness by itself (22). Contrary to these findings Bukkapatnam et al., in 2021 found gain in the keratinized gingival width with an increase in the gingival thickness when they treat gingival recession with SCTG (22).

Regarding AM group, the statistically significant improvement of clinical readings from baseline to six months is probably because the matrix of human amniotic membrane contains abundant growth factors and proteins like keratinocyte growth factor, basic fibroblast growth factor, transforming growth factor-beta, nidogen growth factor, epidermal derived growth factor, collagen types I, III, IV, V, VI, and laminin-5. These growth factors provide a bioactive matrix to facilitate, accelerate wound healing, and provide a natural healing environment (23, 24, 25).

There was a significant increase in the mean of TKG by 0.56 mm in SCTG group and 0.13 mm in AM group after 6 months. It was in accordance with the study made by Kothiwale (26). On the other hand, our results were in contrary to the study made by Rehan et al. who found insignificant results after 18 months follow up period after using AM (27).

The significant improvement regarding TKT in SCTG group compared to AM group since CTG has the genetic message for keratinizing the overlying epithelium (28). So, SCTG is the most effective method to achieve the predictable outcome with a great level of esthetics and extremely stable outcomes thus this procedure is the gold standard while evaluating the effectiveness of other techniques (29). Our results were in accordance with Park, et.al 2023, who had a successful periodontal phenotype modification achieved when a combined procedure using a subepithelial connective tissue graft with bone graft substitutes in the mandibular incisors after orthodontic treatment (30).
CONCLUSION

Both group A and group B showed significant improvement regarding all clinical parameters, from baseline to six months except in WKT. While there was significant improvement regarding TKT in SCTG group compared to AM group at 6 months interval. Hence, SCTG can still be considered the gold standard for biotype modifiers.

RECOMMENDATIONS

Further long-term clinical trials with larger sample size, also, histopathologic studies are recommended to detect the effectiveness of the Amniotic membrane.

Conflict of Interest: no conflict of interest.

REFERENCE


