# EVALUATION OF AMNIOTIC MEMBRANE IN TREATMENT OF CERVICO-FACIAL KELOID SCARS

Sameh M. Hassan[1] *BDS*, Ahmed M. EL Sabbagh[2] *PhD*, Ahmed S. El Mahalawy[2] *PhD*

## ABSTRACT

**INTRODUCTION:** Amniotic membrane is the innermost layer of the placenta consisting of a thick basement membrane and an avascular stromal matrix. It can be used as a graft and as a dressing to promote healing.
**OBJECTIVES:** Evaluation of the use of amniotic membrane in cases with keloids in Cervico-facial region.
**MATERIALS AND METHODS:** A clinical study was conducted on 10 patients with keloids. The sample was selected conveniently to fulfill a list of inclusion and exclusion criteria. Then the selected participants have undergone surgical excision of the scar with placement of amniotic membrane under the skin at the excised site and wound closure by primary intention and amniotic membrane was used as a dressing as well. Follow up and clinical evaluation was performed over a period of 3 months using Vancouver Score Scale.
**RESULTS:** Follow-up data collected 3months after the final treatment revealed decreases in the mean score for the following lesion features. Pigmentation from 1.3 to 1.1; Vascularity from 1.3 to 1.2; Pliability from 3.7 to 2.7 and height from 1.6 to 1.1.
**CONCLUSIONS:** The present study showed that the combination of surgical excision and Amniotic membrane placement can be used as a new alternative in treatment of keloid scars.
**KEYWORDS:** Amniotic membrane allograft, keloid scars, wound healing, Vancouver Score Scale.

---

1. BDS, MS, Faculty of Dentistry, Alexandria University.
2. Professor of Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University.

*Corresponding author:*
*E-mail: samehmohyeldien@gmail.com*

## INTRODUCTION

The skin is considered the largest organ in the human body, it acts as a protective barrier against chemical, light, and heat and pathogenic microorganisms moreover controls the loss of essential body fluids such as blood and water. The skin is not uniform throughout the body as differences between color, texture and thickness are observed depending on the anatomical location. Every square inch of the skin is made up of cells, sweat glands, nerve endings and blood vessels arranged in three layers from the outermost layer to the innermost as follows; epidermis, dermis, and hypodermis (1).

Dermal scarring is a highly dynamic and complex process that comprises several processes, three main phases summarizes these processes into: inflammation, tissue formation and tissue remodeling (2). A scar is produced as a result of disturbance in the wound healing process, scarred tissue shows different biomechanical characteristics, compared to the undamaged normal skin, as scarred tissue is breakable and less functional than normal skin tissue (3).

Scars can be classified into atrophic scars, Raised dermal scars, Hypertrophic scars and Keloid scars. The effect of skin tension, infection, environmental factors, genetic predisposition, sex and hormone levels produce different scar types (4).

Keloids are abnormal raised scars unique to humans, characterized by the excessive deposition of collagen in the dermis and subcutaneous tissues secondary to traumatic or surgical injures. Clinical keloids are defined as dermal fibro proliferative tumors that grow beyond the confines of original wounds and rarely regress over time (5,6). It is strongly suggested that keloids are the result of an incapability to regulate and stop the wound healing process promoted by over activation of the fibroblasts, endorsed by the skin tension generated during the execution of natural body movements (7). It is proposed that skin tension promotes aberrant cell signaling transduction during keloid development and progression (8,9).

Several factors have been proposed to explain the development of keloid scar. No single therapeutic modality is best for all keloids. The location, size, and depth of the lesion; the age of the patient; and the past response to treatment determine the type of therapy used. Prevention is key, but therapeutic treatment of hypertrophic scars and keloids includes surgical and nonsurgical approaches. The Surgical approaches include cryotherapy, excision and laser therapy, while the nonsurgical approaches as ;occlusive dressings, compression therapy, intralesional corticosteroid injections, cryosurgery, excision, radiation therapy, laser therapy, interferon (IFN) therapy, 5-fluorouracil (5-FU), doxorubicin, bleomycin, verapamil, retinoic acid, imiquimod 5% cream, tamoxifen, tacrolimus, botulinum toxin, hydrogel scaffold, and over-the-counter treatments (e.g., combination of hydrocortisone, silicon, and vitamin E) (10).

In this study we proposed another treatment modality by using amniotic membrane. Amniotic membrane is the innermost layer of the placenta consisting of a thick basement membrane and an avascular stromal matrix. It can be used as a graft and as a dressing to promote healing.

The aim of our study is to evaluate the effect of amniotic membrane as an allograft in the treatment of facial hypertrophic keloid scars.

## MATERIALS AND METHODS

Ten patients with keloid scars in cervicofacial region were selected from the outpatient clinic or (recalled patients who have undergone previous operations) of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University.

The clinical part of the study was performed after the approval of Research Ethics Committee, Faculty of Dentistry, Alexandria University. Informed consent was obtained from the patients. The patients received both oral and written information about the study.

**Criteria for patient selection**

All selected patients were confirmed to have clinical and pathological evidence of keloid scar formation under the following selection criteria:

***Inclusion criteria***

- Patients having keloid scars in face and/or neck region, both sexes were included with no age limit and patients who accepted to participate in the study.

***Exclusion criteria***

- Patients suffering from uncontrolled systemic disease (e.g.: uncontrolled diabetes), patients suffering from relevant metabolic, autoimmune diseases (as pemphigus vulgaris/ erythema multiform/ Linear IgA Dermatosis), patients suffering from immunosuppressive disease (e.g. Aids, Lymphoma) or patients subjected to any other previous treatment modality for keloid.

**Materials**

***The amniotic membrane preparation***

The Amniotic membrane has been taken freshly from a donated placenta of a healthy mother, admitted at Elshatby Maternity University Hospital and serological tests for AIDS, Syphilis and hepatitis B and C were done for her. Upon receival of seronegative tests, approval for the donation was accomplished .The placenta was received by caesarian section at the operating room of the maternity hospital.

In the Medical Research Institute – Alexandria University, under sterile aseptic conditions, the placenta was cleaned with balanced sterile saline solution to remove all blood clots. The chorio-amnion was stripped from the placenta and further separation of amnion from the chorion was done by blunt dissection. The separated membrane was washed again properly with sterile normal saline after being rinsed with 0.025% sodium hypochlorite solution where all blood, mucus and debris were completely washed out. Figure (1).

**Figure (1):** Showing Amniotic membrane preparation.

The amniotic membrane was cut into pieces and stored in sterile bottles containing 85% glycerol (Pharma trade co, Egypt) at -80˚C refrigerator to be ready for the time of its use.

**Methods**

Clinical evaluation of the scar was performed using Vancouver scar scale (VSS)(11).

***Vancouver Score Scale VSS***

The VSS assesses 4 variables: pigmentation, vascularity, pliability and height/thickness. (Table 1)

**Table (1):** Showing Vancouver scar scale.

| | |
|---|---|
| **Pigmentation** | |
| 0 | Normal Colour (resembles nearby skin) |
| 1 | Hypopigmentation |
| 2 | Hyperpigmentation |
| **Vascularity** | |
| 0 | Normal |
| 1 | Pink (slightly increased in local blood supply) |
| 2 | Red (significant increase in the local blood supply) |
| 3 | Purple (excessive local blood supply) |
| **Pliability** | |
| 0 | Normal |
| 1 | Supple(flexible with minimal resistance) |
| 2 | Yielding (giving way to pressure) |
| 3 | Firm (solid: inflexible,not easily moved, resistant to manual pressure) |
| 4 | Banding( rope like blanches with extension of scar, does not limit the range of motion) |
| 5 | Contracture permenant shortening of scar producing deformity or distortion, limits range of motion |
| **Height (mm)** | |
| 0 | Normal (flat) |
| 1 | <2 |
| 2 | >2 and <5 |
| 3 | >5 |

***A) Pre-surgical phase***

Twenty four hours before the operation the amniotic membrane was removed from the -80°C refrigerator, allowed to reach room temperature, washed with normal saline and was kept in 480 ml saline containing 1,200,000 IU Penicillin (Pencitard, ACDIMA International Trading, Egypt NCPC North Best Co., Ltd ) in refrigerator at 4˚C.

Immediately before operation the membrane was thoroughly washed from penicillin with normal saline to be ready for application. Figure (2)

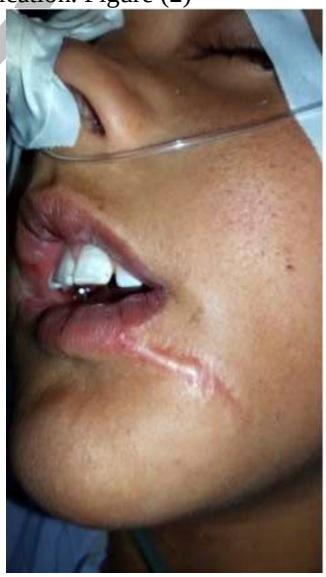

**Figure (2):** Showing the keloid scar preoperatively.

***B) Surgical Phase***

All patients were operated under general anesthesia. The surgical site was scrubbed using povidone–Iodine. The keloid scar was elliptically excised using Bard Parker blade number 10 and undermining of the free margins using dissecting scissors for proper tension free wound closure.

The prepared amniotic membrane was placed with its inner side toward the dermis and sutured with 4-0 vicryl.

Skin subcuticular sutures were done using 6-0 proline to allow proper closure of wound over the fixed amniotic membrane, another piece of the amniotic membrane was used as a biologic dressing and sutured over the operation site for one week. Figure (3)

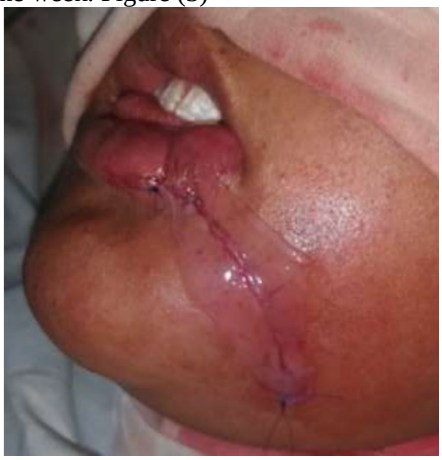

**Figure (3):** Showing sutured wound after keloid scar excision with the biologic dressing on it.

***C) Postsurgical phase***

**1. Postoperative Care**

Cold fomentation at 3 minutes interval every 30 minutes in the first postoperative 24 hours, followed by hot fomentation to the end of the week. Patients were instructed to avoid touching or manipulating the dressing. The biologic dressing was removed one week postoperatively.

**2. Postoperative Medications.**

Antibiotic in the form of Amoxicillin 875+Clavulanic acid 125 (Augmentin 1gm tablets, GlaxoSmithKline). Adults dosage is 1 tablet every 12 hours for 7 days, Children under 12 years 20-40 mg/Kg body weight divided in 2-3 doses daily for 7 days.

Adults Analgesic/anti-inflammatory Diclofenac potassium 50 mg tablets (Cataflam, Novartis, Egypt) 1 tablet every 12 hours for 5 days. Children: Diclofenac Potassium suspension (Catafly, Novartis, Egypt) suspension1mg-3mg divided in doses daily

***D) Follow up phase***

**Clinical evaluation**

The wound was evaluated for signs and symptoms of infection including redness, hotness, swelling and /or discharge.

The evaluation was performed using the Vancouver Scar Scale (VSS) after one week and 3 months postoperatively. Figure (4)

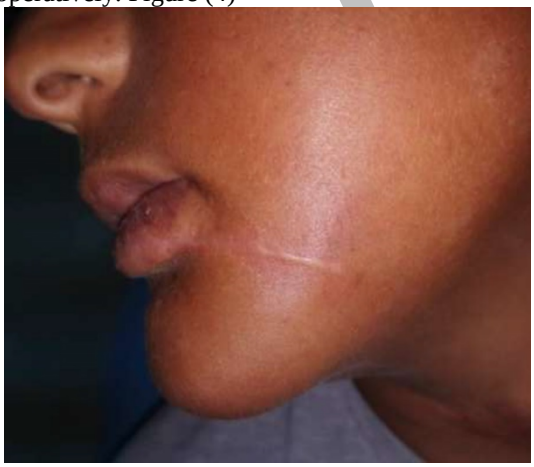

**Figure (4):** Showing the patient three months post operatively.

Data were fed to the computer using IBM SPSS software package version 21.0.Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using median, minimum and maximum. For normally distributed data, comparison between two independent populations was done using independent t-test.

## RESULTS

Ten patients (6 males and 4 females) with keloid scars were attended in this study .The mean age was 10.5 years. Site of keloid scars were lower lip (n=4), neck (n=4) and cheek (n=2). The etiologic factor of keloid scar was either trauma (n=6) or incision of surgical approach from a previous surgery (n=4).

**Preoperatively the data collected revealed**

Pigmentation from (range 1–2), vascularity from (range 0–2), pliability from (range 1–2) and height from (range 6–10).

After 3 months postoperatively the data collected revealed the following:

- Pigmentation from (range 1–2) to (range 1–2) (*P* = 0.144) no statistical significance
- Vascularity from (range 0–2) to 1 (range 0–2) (P = 0.368) with no statistical significance
- Pliability from (range 1–2) to (range 0–2) (P= 0.027) with statistical significance height from (range 6–10) to (range 4–8) (*P* = 0.004) with statistical significance.

The VSC, the sum of individual component scores, decreased from (range 6–10) to (range 4–8) (*P=0.004*) statistically significant. Table (2)

**Table (2):** Showing the comparison between the pre and post-operative results of the study.

| | Pigmentation | | Vascularity | | Pliability | | Height | | Total Score | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PRE | POST | PRE | POST | PRE | POST | PRE | POST | PRE | POST |
| Range | 6-10 | 1-2 | 0-2 | 0-2 | 3-4 | 2-4 | 1-2 | 0-2 | 6-10 | 4-8 |
| Mean | 8.0 | 1.1 | 1.3 | 1.2 | 3.7 | 2.7 | 1.6 | 1.1 | 8.0 | 6.0 |
| SD | 1.56 | 0.32 | 0.67 | 0.63 | 0.48 | 0.67 | 0.52 | 0.57 | 1.56 | 1.49 |
| t-test | 1.36 | | 0.925 | | 4.22 | | 2.01 | | 3.85 | |
| p | 0.144 N.S. | | 0.368 N.S. | | 0.001 | | 0.027 | | 0.004 | |

The pigmentation and the vascularity were found to be statistically non-significant, while pliability, height and total score were found to be of statistical significance with regards to Vancouver scar scale.

## DISCUSSION

Many studies on wound healing stages have found abnormal changes in the remodeling stage, and the main change was the imbalance in collagen production and degradation, which was hypothesized to be the cause of keloid scar formation.(2)

In the present study the age group was found to be in young patients with age ranged from 6 -15 years which is in agreement with Berman and colleagues in 2007 (12), who studied the biologic effects, clinical efficacy, and safety of silicone elastomer sheeting for hypertrophic and keloid scar treatment and management. They concluded that keloids were more common in persons younger than 30 years, with risk peaking between 10 to 20 years of age. This seems to be due to elevated hormonal levels in patients (e.g., during puberty or pregnancy).(12)

There was no difference in keloids occurrence in different sexes where it was found that male to female

incidence was 3:2 respectively this was in accordance with Verhaegen et al in 2009 .(13)

The most common sites in the cervico facial region in the present study were lower lip as a result of trauma (N=4) and the neck region as a result of previous operation (N=4). Studies reporting the treatment of keloid in the cervicofacial region only were scarce and the cervicofacial keloids were reported to be more common in the cheek as investigated by Burd and Huang in 2005 (14), However in the present study only 2 cases were found to be in the cheek area.(14)

The reason for prevalence of the keloid in certain sites can be explained by the presence of relaxed skin tension lines which when crossed causes apparent scar formation.

In the present study the amniotic membrane was used as an allograft beneath the skin as well as a biological dressing over the wound which was in contrast with Mohammadi and colleagues (15), who used it as a dressing only to evaluate the possible role of amniotic membrane as an adjunct to split thickness skin grafting on reducing severity of hypertrophic scar formation in post burned patients. (15).

In the present study the total VSS scores were reported to decrease from (6-10) to (4-8), this agrees with Cho and colleagues in 2010 (16), studied the efficacy and safety of 1064-nm Q-switched Nd: YAG laser with low influence for keloids and hypertrophic scars have used the same scar scale score as the present study (VSS) and reported improvements of pigmentation, vascularity, pliability, and scar height after five to ten treatment sessions (at 1–2-week intervals). Using VSS score, their study showed that the sum of individual component scores, decreased from 8 (range 6–12) to 6 (range 3–8). Although different treatment methods were used in this study and the present study, the results showed to be very close and this shows how keloids are challenging to different treatment modalities (16).

The goal of the current study was to excise the previously formed keloid scars of the cervicofacial region and repair the area with suturing and application of pre prepared amniotic membrane beneath the skin and another grafted piece of the amniotic membrane to act as a biologic dressing over the skin.

Amniotic membrane as an allografting material is a novel modality in treatment of keloid scars, which reduces scar formation. However more studies need to be carried out to prove its efficiency in treatment of keloid scars or its use as a safe cheap allograft.

Surgical excision of keloids is associated with recurrence rates varying from 40 to 100%, therefore we have combined in our study excision of the keloid scar with another treatment modality as amniotic membrane placement ,this concept was supported by Mustoe in 2008 (17),Simple excision is thought to promote additional collagen synthesis, resulting in fast regrowth and frequently an even larger keloid (17).

## CONCLUSION

The Amniotic membrane can be a new alternative in treatment of Keloid scar, it has the advantage of being a cheap available allograft.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES


1. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol. 2002;12:390-9;quiz 400-1.
2. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37:1528-42.
3. Van Zuijlen PP, Ruurda JJ, van Veen HA, van Marle J, van Trier AJ, Groenevelt F, et al. Collagen morphology in human skin and scar tissue: no adaptations in response to mechanical loading at joints. Burns. 2003;29:423-31
4. Sommerlad BC, Creasey JM. The stretched scar: A clinical and histological study: BC Sommerland and JM Creasey. Br J Plast Surg. 1979;31:26–28.
5. Butler PD, Longaker MT, Yang GP. Current Progress in Keloid Research and Treatment. J Am Coll Surg. 2008r;206:731-41.
6. Tuan TL, Nichter LS. The molecular basis of keloid and hypertrophic scar formation. Mol Med Today. 1998;4:19-24.
7. Alenghat FJ, Ingber DE. Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. Sci STKE. 2002;2002:e6.
8. Suarez E, Syed F, Alonso-Rasgado T, Mandal P, Bayat A.. Up-regulation of tension- related proteins in keloids: Knockdown of hsp27, α2β1-integrin, and pai-2 shows convincing reduction of extracellular matrix production. Plast Reconstr Surg. 2013;131:158e-73e.
9. Wong VW, Rustad KC, Akaishi S, Sorkin M, Glotzbach JP, Januszyk M, et al. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. Nat Med. 2011;18:148-52.
10. Poetschke J, Gauglitz GG, Current options for the treatment of pathological scarring, J Dtsch Dermatol Ges 2016;14:467-77.
11. Fearmonti R, Bond J, Erdmann D, Levinson H. A Review of Scar Scales and Scar Measuring Devices. Eplasty. 2010;10:e43.
12. Berman B, Perez OA, Konda S, Kohut BE, Viera MH, Delgado S, et al. A review of the biologic effects, clinical efficacy, and safety of silicone elastomer sheeting for hypertrophic and keloid scar treatment and management. Dermatol Surg. 2007;33:1291-303.
13. Verhaegen PD, van Zuijlen PP, Pennings NM, van Marle J, Niessen FB, van der Horst CM, et al. Differences in collagen architecture between keloid, hypertrophic scar, normotrophic scar, and normal skin: An objective histopathological analysis. Wound Repair Regen. 2009;17:649-56.
14. Burd A, Huang L. Hypertrophic response and keloid diathesis: two very different forms of scar. Plast Reconstr Surg 2005;116:150e-7e.
15. Mohammadi AA, Eskandari S, Johari HG, Rajabnejad A. Using Amniotic Membrane as a Novel Method to Reduce Post-burn Hypertrophic Scar Formation: A Prospective Follow-up Study. J Cutan Aesthet Surg. 2017;10:13-7.
16. Cho SB, Lee JH, Lee SH, Lee SJ, Bang D, Oh SH. Efficacy and safety of 1064-nm Q-switched Nd:YAG laser with low fluence for keloids and hypertrophic scars. Eur Acad Dermatol Venereol. 2010;24:1070-4.
17. Mustoe TA. Evolution of silicone therapy and mechanism of action in scar management. Aesthetic Plast Surg. 2008;32:82-92.

18. Choi Y-H, Kim K-M, Kim H-O, Jang Y-C, Kwak I-S. Clinical and Histological Correlation in Post-Burn Hypertrophic Scar for Pain and Itching Sensation. Ann Dermatol. 2013;25:428-33.