

## Utilization of amniotic membrane graft for repair of the tympanic membrane perforation

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### Abstract

**Introduction:** Myringoplasty is necessary to control infection, protect the middle ear and restore hearing. Amniotic membrane, which used widely in grafting of the conjunctiva, supposed to be the new tympanic membrane graft. This study carried out to evaluate the utilization of amniotic membrane graft versus temporalis fascia graft in myringoplasty. **Materials & Methods:** Sixty-four patients with the diagnosis of chronic suppurative otitis media without cholesteatoma undergo myringoplasty divided randomly into 2 groups: - the control group (temporalis fascia group, TFG) and the study group (the amniotic membrane group, AMG). All patients subjected to detailed medical history, complete ENT examinations and audiological test including pure tone audiometry (pre & postoperative). **Results:** The mean operation duration for the temporalis fascia group was  $75.3 \pm 9.4$  minutes, while was  $54.3 \pm 8.3$  minutes for the amniotic membrane group which shows a statistically significant lower than temporalis fascia group ( $p < 0.01$ ). The overall healing success rate in the TFG group was 87.5%, while 84.4% in AMG group without any significant difference between both groups. **Conclusion:** Utilization of amniotic membrane graft in myringoplasty shows similar outcome to temporalis fascia graft.

*Key words:* amniotic membrane graft, otitis media, temporalis fascia, myringoplasty.

### Introduction:

Myringoplasty is a necessary procedure to control infection, protect the middle ear and restore hearing mechanism<sup>1</sup>. Usually autologous grafts from patient's own tissues are used to reconstruct the tympanic membrane perforation<sup>2</sup>.

The closure of tympanic membrane perforations and elimination of chronic drainage are achieved effectively when performing myringoplasty with temporalis fascia<sup>3</sup>. However, a failure rate over 10% of patients undergoing myringoplasty with temporalis fascia where graft can occur<sup>4</sup>. In addition, post auricular incision is mainly for graft taken which increase the operative time<sup>2</sup>.

The amniotic membrane, or amnion, comprises the innermost layer of the placenta. The amniotic membrane consists of a single layer of cuboidal epithelial cells, a thick basement membrane and an avascular stromal matrix, loosely attached to the chorion<sup>5</sup>. The amniotic membrane transplantation (AMT) has been used in many different types of reconstructive surgery<sup>6</sup>.

Amniotic membrane, which used widely in grafting of the conjunctiva, supposed to be the new tympanic membrane graft. AMT has the ability to diminish the

occurrence of adhesions, scarring, enhance wound healing, reduce inflammation and maintain a normal epithelial phenotype and epithelialization. Its antimicrobial potential expresses incomplete HLA-A, B, C, and DR antigens<sup>7,8</sup>.

There was no immunological rejection after transplantation observed after use of a fresh fetal membrane (i.e. both amnion and chorion) as a graft for conjunctiva surface reconstruction with limited success. Interest in AMT then waned and it was not until Kim and Tseng, 1995, successfully reintroduced the concept and interest in AMT revived<sup>9</sup>.

This study was carried out to evaluate the utilization of amniotic membrane graft versus temporalis fascia graft in myringoplasty.

### Materials & Methods:

A Prospective study carried out in the Otolaryngology department of Suez Canal University hospital, Ismaili, Egypt. Sixty-four patients, age between 18 -50 years old, with the diagnosis of chronic suppurative otitis media without cholesteatoma underwent myringoplasty. Patients with active infection, cholesteatoma, previous ear surgery and /or refuse for surgery were excluded from the study.

All patients were subjected to complete ENT detailed history and examination and audiological tests (pure tone audiometry and ET functions). Patients were divided randomly into 2 groups: the control group: temporalis fascia group (TFG) and the study group: the amniotic membrane group (AMG). Postoperative follow up to detect any infection or complication at the following

intervals: 1st, 2nd ,4th weeks, 3rd and 6th months with finalre evaluation by audiological test .

*Technique of myringoplasty:* Under general anesthesia, a post auricular approach was used and underlay tympanic membrane grafting technique performed for both groups with absorbable gel foam placed under grafted drum to support of the graft. The temporalis fascia graft taken from the same operating ear side for TFG.

For the AMG, the amniotic membrane is harvested from consenting seronegative (hepatitis B, C virus, syphilis and human immunodeficiency virus) maternal donors during elective Caesarian section under sterile conditions. The placental membrane was washed in a balanced salt solution (BSS) to remove clots and debris. The membrane was then bathed in a cocktail of antimicrobial medium containing penicillin, streptomycin, neomycin, and amphotericin-B for 24 hours, followed by a second wash in BSS. The amnion separated from the chorion, divided into pieces measuring approximately 1cm, and mounted, stoma side down, onto nitrocellulose cards. The membrane was placed in a plastic container, and stored in 50% glycerol at -80°C. All amniotic membranes presented a negative result in the microbiological cultures. The membrane was defrosted immediately before use by warming the container to room temperature for 10 minutes. A small piece of membrane was taken and washed by normal saline before use (Figure 1).

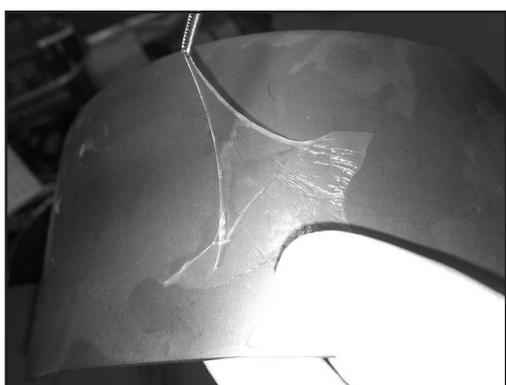


Figure 1: Amniotic membrane during preparation for surgery.

**Results:**

There were 32 patients in each group. Females represent 56.3% of the patients while males were 43.7%. The mean age for the patients in TFG was 29.7±8.8 years, while the mean age was 30.1±8 years in AMG.

The main patients complaint in both groups was recurrent ear discharge and conductive hearing loss of different degrees (98.4% and 100%, respectively). Tinnitus was found in 80%, while 76.6% were complaining of earache. Headache and vertigo were the least presenting symptoms (Table 1).

|               | Temporalis fascia group (n=32) |      | Amniotic membrane group (n=32) |      | Total (n=64) |       | p value |
|---------------|--------------------------------|------|--------------------------------|------|--------------|-------|---------|
|               | No.                            | %    | No.                            | %    | No.          | %     |         |
| Ear discharge | 31                             | 96.9 | 32                             | 100  | 63           | 98.4  | 1.00    |
| Hearing loss  | 32                             | 100  | 32                             | 100  | 64           | 100.0 | 1.00    |
| Tinnitus      | 25                             | 78.1 | 26                             | 81.3 | 51           | 79.7  | 0.99    |
| Earache       | 24                             | 75.0 | 25                             | 78.1 | 49           | 76.6  | 0.98    |
| Vertigo       | 1                              | 3.1  | 2                              | 6.3  | 3            | 4.7   | 0.88    |
| Headache      | 3                              | 9.4  | 4                              | 12.5 | 7            | 10.9  | 0.79    |

Table 1: Main presenting symptoms in study groups. Not Significant if p-value > 0.05.

The mean operative duration in the TFG group was 75.3±9.4 minutes, while the mean time was 54.3±8.3 minutes in AMG group, which was statistically different (Table 2).

|   | Temporalis fascia group (n=32) | Amniotic membrane group (n=32) | Total (n=64) | p value |
|---|--------------------------------|--------------------------------|--------------|---------|
| <b>Duration of operation (minutes):</b> |                                |                                |              |         |
| Mean± SD                                | 75.3±9.4                       | 54.3±8.3                       | 3.4±2.2      | 0.000** |
| Range                                   | 45-90                          | 40-75                          | 1-10         | -       |

Table 2: Duration of operation in study groups  
\*\* Significant

Success rate in the TFG group was 87.5%, while 84.4% in AMG group without any statistically differences (small size perforations show success rate 100%, large perforations success rate 75% while most of the failure rates occurred in subtotal perforations 50% in both groups)(Table 3).

|          |         | Temporalis fascia group (n=32) |      | Amniotic membrane group (n=32) |      | p value |
|----------|---------|--------------------------------|------|--------------------------------|------|---------|
|          |         | No.                            | %    | No.                            | %    |         |
| Small    | Success | 6                              | 100  | 5                              | 100  | 1.00    |
|          | Failure | 0                              | 0    | 0                              | 0    |         |
| Medium   | Success | 15                             | 93.8 | 15                             | 88.2 | 0.87    |
|          | Failure | 1                              | 6.2  | 2                              | 11.8 |         |
| Large    | Success | 6                              | 75.0 | 6                              | 75.0 | 1.00    |
|          | Failure | 2                              | 25.0 | 2                              | 25.0 |         |
| Subtotal | Success | 1                              | 50.0 | 1                              | 50.0 | 1.00    |
|          | Failure | 1                              | 50.0 | 1                              | 50.0 |         |
| Overall  | Success | 28                             | 87.5 | 27                             | 84.4 | 0.76    |
|          | Failure | 4                              | 12.5 | 5                              | 15.6 |         |

Table 3: Success and failure rates of graft taken according to the size of perforation in both groups. Not Significant if p value > 0.05.

The mean Air/Bone gap preoperatively in the TFG group was 37.3±6.3 dB, while 37.9±3.8 dB in AMG group. Postoperative shows marked improvement as 13.7±4.6 dB Air/Bone gape in TFG group, while 14.1±2.3 dB in AMG group without any statistically significant differences between both groups (Table 4).

|   | Temporalis fascia group (n=32) | Amniotic membrane group (n=32) | Total (n=64) | p value |
|---|--------------------------------|--------------------------------|--------------|---------|
| <b>Preoperative Air Bone Gap (db):</b>  |                                |                                |              |         |
| <b>Mean± SD</b>                         | 37.3±6.3                       | 37.9±3.8                       | 38.5±5.2     | 0.55    |
| <b>Range</b>                            | 25-50                          | 26-52                          | 25-52        | -       |
| <b>Postoperative Air Bone Gap (db):</b> |                                |                                |              |         |
| <b>Mean± SD</b>                         | 13.7±4.6                       | 14.1±2.3                       | 15.8±6.4     | 0.43    |
| <b>Range</b>                            | 10-20                          | 10-21                          | 10-21        | -       |

Table 4: Mean Air/bone gap pre and post-operative in study groups Not Significant if p-value > 0.05.

Wound infection occurred in 12.5% of TFG group, while infection occurred in 9.4% in AMG group. Postoperative otorrhea occurred in 6.3% in TFG group, and in 9.4% of AMG group without any statistically significant difference.

### Discussion:

Chronic perforations of the eardrum or tympanic membrane represent a significant source of morbidity worldwide<sup>10</sup>. Myringoplasty is a simple repair of the tympanic membrane perforation without ossicular reconstruction<sup>2</sup>. This procedure is commonly performed by otolaryngologists to improve hearing and limit patient susceptibility to middle ear infections<sup>10</sup>.

Skin grafts were first used with high failure rates and cholesteatoma formation<sup>11</sup>. Temporalis fascia was later established as graft material for tympanic membrane reconstruction, with a failure rate over 10%<sup>12</sup>.

The amniotic membrane, or amnion, which is the internal "layer" of the placenta, was used for ocular surface reconstruction<sup>7,8,13</sup>. The amniotic membrane has a very weak antigenicity that severe immune responses after grafting have never been described without any significant rejection<sup>13</sup>. In addition, it has an antimicrobial property, reducing the risks of postoperative infection and promoting cell migration/growth activity<sup>14</sup>. The amniotic membrane also can be frozen and available for use at least for 12 months<sup>15</sup>. It has been used with good results as temporary or long term wound dressing, vaginal epithelization, and replacement of nasal mucosa in hereditary hemorrhagic telangectasia and corneal ulceration<sup>16</sup>.

The main complaint among our study population was ear discharge (98.4%) and conductive hearing loss (100%). Similarly, Ibrahim et al, 2002, found that the main presenting symptom was deafness (100%) and ear discharge in 61.6% of his study population<sup>17</sup>.

The main failure causes were due to postoperative infection (4 cases in TFG group and 3 cases in AMG group). Harvinder et al, 2005, found that success rate for the amniotic membrane graft was 65% while 56.7% for the temporalis fascia graft<sup>14</sup>.

Fishman et al, 2005, mentioned that Alloderm as a grafting material has 84% success rate while temporalis fascia graft success rate about 97<sup>18</sup>. While Ibrahim et al, 2002, investigated temporalis fascia graft in myringoplasty with a success rate of 75%<sup>17</sup>.

Singh et al, 2003, demonstrated 93.3% closure rate using an overlay and underlay technique in 60 patients with dry subtotal perforations<sup>19</sup>. Khan et al, 2002, reported a tympanic membrane closure rate of 84% at 5 year follow-up with an underlay tympanoplasty using temporalis fascia<sup>20</sup>. Berger et al, 1997, found a success rate of 52.8% with onlay technique in revision myringoplasty patients. The advantages of the temporalis fascia include the ease of harvest from the same incision as the operation, availability of a large amount of graft, and very high take rates without subsequent problems<sup>21,22</sup>.

Many studies reported the successful tympanoplasty using temporalis fascia grafting. The success rate ranged from 75% to 96.7%<sup>1,3,4,23-30</sup>. The main outcome measures were the graft take rate and postoperative hearing gain.

Human amniotic membrane has been used with good results since Davis first reported the use of fetal membrane in skin transplantation in 1910<sup>31</sup>. Human amniotic membrane used instead of synthetic materials to avoid adhesions and reactions. Its antigenicity appears to be very low with no evidence of rejection after transplantation<sup>32</sup>.

Amniotic membrane showed the ability to decrease bacterial growth. Human amniotic membrane is readily available, easily stored and inexpensive. It is used with good results as replacement of nasal mucosa in hereditary hemorrhagic telangectasia, arthroplasty and in ear surgery to close the small tympanic membrane perforations and as lining in mastoid cavities with an 84.8% success rate<sup>33</sup>.

Our study showed that there was no statistically significant difference between the mean Air/Bone gap in both groups. Similar results were observed in the study of Harvinder et al, 2005, with temporalis fascia and amniotic membrane grafts resulting in significant closure of Air/Bone gap<sup>14</sup>.

Regarding audiometric evaluation, Fishman et al, 2005, reported that the overall postoperative air-bone gap was less than or equal to 20 dB in 89% of all patients undergoing temporalis fascia grafting. Sixty-six percent of patients achieved an air-bone gap of less than or equal to 15 dB. Fifty-one percent had an ultimate air-bone gap of 10 dB or less<sup>18</sup>.

Ibrahim et al, 2002, found that there is a significant statistical difference between pre and postoperative air conduction in temporalis fascia grafting at all measured frequencies. Closure of Air/Bone gap to within 0-10 occurred in 70% of patients<sup>17</sup>.

Postoperative improvement in hearing level reported using temporalis fascia ranged from 9 to 18dB<sup>1,3,4,23-30</sup>. In conclusion, utilization of amniotic membrane graft in myringoplasty shows similar outcome to temporalis fascia graft.

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