

Role of Stem Cells in Healing of Tympanic Membrane Perforations

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Abstract

Background: Stem cells are biological cells found in all multicellular organisms, that can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. Currently, surgery is the treatment of choice for repairing the perforation. However, surgery is time-consuming and expensive, and there is a risk of complications. Repairing a perforation by office techniques has been successful for a few cases for small traumatic perforations.

Aim of Study: In this study we aimed to study the role of stem cell therapy in management of tympanic membrane perforations by otoscopic examination, microscopic, endoscopic examination and audiological assessment.

Patients and Methods: Thirty patients included in our prospective study in Al-Zahra University Hospital and Shebin El-Kom Teaching Hospital from April 2014 up to December 2018. These patients were operated for simple myringoplasty with stem cell technique. These patients followed-up with otomicroscopic examination of the perforation, tympanometry and pure tone audiometry.

Results: There were female patients much more than male patients. The mean age of the patients was 31.9 ± 8.2 years. There is highly significant improvement in the air bone gap and ear canal volume differences before and 3 months after the operation. Doctor satisfaction was 96.7% while patient satisfaction was 94.1%. 96.7% of cases had successful results of perforation closure. Of 30 patients operated by stem cells therapy under complete aseptic techniques 29 patients TM perforations had completely healed after first trial (success rate 96.7%) and one case failed (failure rate 3.3%).

Conclusion: Stem cells therapy is a new effective technique more safer successful, economic, easier to operate and time consuming than the traditional tympanoplasty. When the perforation is small to medium size maximum 4 X 4mm and dry since more than one month. This technique is better and to avoid complications and side effects of general anaesthesia.

Key Words: Audiometry – Tympanic membration perforations – Stem cells therapy – Tympanometry – Tymapanoplasty and Stem cells therapy.

Introduction

STEM cells are biological cells found in all multicellular organisms, that can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. In mammals, there are two broad types of stem cells: Embryonic stem cells, which are isolated from the inner cell mass of blastocysts and adult stem cells, which are found in various tissues [1].

In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells (these are called pluripotent cells), but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues [2].

Unipotent cells can produce only one cell type, their own, but have the property of self-renewal, which distinguishes them from non-stem cells (e.g., muscle stem cells). Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells. Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers [3].

Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. Bone marrow has been found to be one of the rich sources of adult stem cells which have been used in treating several conditions including peripheral nerve injuries (e.g. facial nerve palsy), spinal cord injury,

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liver cirrhosis, chronic limb ischemia and end-stage heart failure [4].

The bone marrow stem cell quantity has been found to be declining with age and in reproductive age group of females it is relatively lesser than in males of same age group. A great deal of adult stem cell research to date has had the aim of characterizing the capacity of the cells to divide or self-renew indefinitely and their differentiation potential. In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. Unfortunately, many mice do not live long with stem cell organs [5].

Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin e.g. mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc [6].

Most acute Tympanic Membrane (TM) perforations heal spontaneously but a few develop into a chronic stage. Chronic TM perforation causes draining ears and hearing impairment. In developing countries, medical treatment of TM perforations is often not accessible. Currently, surgery is the treatment of choice for repairing the perforation. However, surgery is time-consuming and expensive, and there is a risk of complications. Repairing a perforation by office techniques has been successful for a few cases for small traumatic perforations [7].

Our aim of this study is to study the role of stem cell therapy in management of tympanic membrane perforations by otoscopic examination, microscopic examination and audiological assessment.

Patients and Methods

This study was done on thirty patients of perforated drum presented to Otorhinolaryngology Departments of Al-Zahra University Hospital and Shebin El-Kom Teaching Hospital and after approval of the Ethical Committee of the hospitals. After consent taken from all patients who underwent extensive pre-operative evaluation in the form of history taking, physical examination, complete laboratory investigations and documentation. Bone marrow samples will be aspirated from anterior iliac crest of every patient under local anesthesia injection of 2-3ml lidocaine. Mononuclear cells will be separated by density gradient layering then cultivated in 4-2-hydroxyethyl-1-piperazineethanesulfonic (HEPES) acid tissue culture and incubated in CO₂ incubator at 37°C was done. After

36-48 hours the adherent cells was separated using trypsin and collagenase. The adherent stem cells were dispersed in normal saline in concentration of 1.2 per ml and then 3-4cm of pure stem cells fluid which is glue like fluid was administered direct locally after putting gel foam in the middle ear spreading the stem cells fluid on the perforation all around and inside the middle ear and outside the middle ear in the canal the putting gel foam overlying the stem cells fluid (after preparing the drum perforations by application of hydrocortisone for 10 days and refreshing of the edges) on the tympanic membrane perforation. Follow-up of the patients by short term study (twice per week for 14 days) and long term study (after 3 and 6 months) by otoscopic examination, microscopic examination and audiological assessment. Analytic studies and comparisons are done and tabulated according to age, sex, site, laterality, Pure Tone Audiometry (PTA), tympanometry, Air Bone Gap (ABG), ear canal volume, doctor satisfaction and patient satisfaction) all items are compared before and 3 months after the operation.

Results

Female patients are much more than male patients. Mean age of patients was 31.9 years old as shown in (Table 1).

Mean pre-operative ABG was 25 but with highly significant improvement with closure of the ABG was shown in post-operative results after 3 months as shown in (Table 2).

20 cases with central perforation had successful closure of tympanic membrane perforation while only one case still had perforated tympanic membrane. All 4 cases with anterior perforation had successful results. Also all 6 cases with posterior perforation had successful closure of tympanic membrane perforation.

Regarding the outcome:

Majority of patients (94.1%) were very satisfied. The majority of cases (96.7%) were satisfied by the operated doctor as shown in (Table 4).

Table (1): Epidemiological study of dry CSOM cases.

| Variables | Patients with dry CSOM=30 | |
|----------------|---------------------------|------|
| Age (years) | 12-51 years old | |
| Mean ± SD | 31.9±8.2 | |
| <i>Gender:</i> | No. | % |
| Male | 10 | 33.3 |
| Female | 20 | 66.7 |

Table (2): Pre-operative and post-operative (ABG).

| | Patients with CSOM=30 Mean ± SD | t-test | p-value |
|---|------------------------------------|--------|---------|
| • Pre-operative ABG | 25±3.1 | 6.315 | 0.00001 |
| • Post-operative ABG- after 3 months | 0 | | HS |

p-value: NS = Non-significant (p-value >0.05).
S: Significant (p-value ≤0.05).
HS: Highly significant (p-value ≤0.001).

Table (3): Healing after stem cells therapy results according to site of perforation.

| Healing results | Cases with failed closure=1 | | | | Chi-square test | p-value |
|-----------------------|-----------------------------|------|--------|-----|-----------------|---------|
| | Successful | | Failed | | | |
| | No. | % | No. | % | | |
| Central perforation | 19 | 66.7 | 1 | 3.3 | 2.786 | 0.594 |
| Anterior perforation | 4 | 13.3 | 0 | 0 | | |
| Posterior perforation | 6 | 20 | 0 | 0 | | |
| The total results | 29 | 96.7 | 1 | 3.3 | | |

p-value: NS = Non-significant (p-value >0.05).
S = Significant (p-value ≤0.05).
HS = Highly significant (p-value ≤0.001).

Table (4): Satisfaction of doctor and patient in cases of CSOM.

| Satisfaction score | No. of CSOM cases=30 Mean ± SD |
|--------------------|-----------------------------------|
| Doctor | 96.7±18.3 |
| Patient | 94.1±1.9 |

Discussion

Recent advances in developmental biology and tissue engineering provide the opportunity to repair damaged or lost tissues with cells supplied from exogenous sources. The therapeutic use of stem cells is gaining a great momentum; it is believed to be the gold mine of science. These cells are implicated in the healing of wounds in general due to their proved regenerative capacities [8].

Von Unge et al., in 2002 tested the healing capacity of stem cells in fresh tympanic membranes in Mongolian gerbils. Functional and morphological measures of the TMs were assessed. In this study, 5 adult Mongolian gerbils were used in a Moire interferometry group and 9 were used in a fluorescence microscopy group. There were better and higher healing scores in the stem cell treated groups [9].

In our study, female patients are significantly much more than male patients. Mean age of patients was 31.9 years old. Mean preoperative air bone

gap was 25 and showed complete closure after 3 months follow-up.

The most striking finding in this study is the high rate of perforation closures (96.7%) in the ears treated with stem cells. But there is no statistically significant difference between results of healing of the perforation among studied groups according to site of perforation.

Doctor satisfaction according to the 5 following parameters 20% each:

- Complete healing of the perforation after 3 months of the operation.
- ABG closure 3 months post-operative.
- Improvement of the ear canal volume to be in the normal range (1.25 to 1.75).
- Improvement of the tympanometry to be normal type (a) 3 months post-operatively.
- Improvement of the PTA to be normal 3 months after the operation.

Doctor satisfaction according to previous criteria was 96.7%. Patient satisfaction (subjective) was 94.1%.

Conclusion

Stem cell is a new effective technique in simple myringoplasty. When the perforation is small to medium size maximum 4 X 4mm and dry since one month at least it is recommended to do stem cells therapy as it is safer. This technique is better, more economic, more successful results, easier to operate and to avoid complications and side effects of general anesthesia.

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دور الخلايا الجذعية فى إلتئام ثقبوط طبلة الأذن

إن الخلايا الجذعية هى خلايا حيوية توجد فى الكائنات متعددة الخلايا وهى قادرة على تجديد نفسها من خلال أنقسام ميتوزى وتشكل إلى مختلف الخلايا المتخصصة وأيضاً تنتج خلايا جذعية جديدة. الخلايا الجذعية فى الثدييات تنقسم إلى نوعين خلايا جذعية جنينية وتنشأ من الكتلة الخلوية الداخلية حيث يمكن عزلها وخلايا جذعية بالغة وتوجد فى مختلف الأنسجة.

تعد الخلايا الجزعية وخلايا المنشأ هى الوسيلة للإصلاح والتجديد فى الكائنات البالغة إلى مختلف الأنسجة البالغة. بالنسبة للخلايا الجذعية الجنينية فإنها قادرة على الأنقسام إلى كل أنواع الأنسجة المتخصصة مثل الدم والأنسجة المعوية والجلد وخلافه كما أنها تحافظ على عملية الأكل والتجديد الطبيعية.

إن الخلايا أحادية الإخصاب قادرة على الأنقسام وتجديد نفسها إلى نوع واحد فقط من الخلايا مثل الخلايا العضلية فقط.

أما الخلايا متعددة الإخصاب من نوع واحد فهى قادرة على الأنقسام إلى أنسجة متعددة من نفس العائلة من إحدى طبقات الجسم.

أما الخلايا متعددة الإخصاب فهى قادرة على الأنقسام إلى مختلف أنواع الأنسجة من طبقات الجسم الثلاثة.

إن الخلايا الجذعية متعددة الإخصاب إلى مختلف الأنواع من الأنسجة نادرة وتوجد فى الحبل السرى للجنين وفى نخاع الشوكى حيث تمثل مصدراً غنياً للخلايا البالغة فى حالات إصابات العصب الطرفية مثل العصب الوجهى السابع وإصابات النخاع الشوكى وتليف الكبد وفشل القلب الحاد وخلافه.

إن الخلايا الجذعية من نخاع الشوكى تقل تدريجياً مع تقدم العمر وهى أقل فى السيدات فى عمر الإخصاب من الرجال فى نفس العمر. هذا المجال بحاجة إلى بحث مكثف لمعرفة قدرة الخلايا الجذعية على تجديد النفس والقدرة على التنوع إلى مختلف الأنسجة.

فى الفئران تمثل الخلايا العظمية مصدراً للخلايا الجذعية متعددة الإخصاب إلى أنسجة متنوعة ومن سوء الحظ فإن الفئران لا تعيش طويلاً بدون أعضاء غنية بالخلايا الجذعية.

إن معظم ثقبوط طبلة الأذن تلتئم تلقائياً ولكن القليل منها يتطور إلى مرحلة مزمنة ويؤدى ذلك إلى إفرازات من الأذن وإعاقة بالسمع وفى الدول النامية فإن العلاج الدوائى غالباً لا يكفى فقد أصبحت الجراحة حديثاً هى الحل الأمثل ولكن الجراحة تستنزف الوقت والمال ولها مضاعفات ولذلك فإن الطرف المكتنبة أصبح لها دور فى بعض الحالات خاصة الثقبوط الصغيرة الناتجة عن إصابات الأذن.

أقر العلماء مثل كروز وآخرون أن الخلايا الجذعية لها دور فى الأنقسام إلى خلايا الطبقة السطحية من قشرة الجلد والتي كانت توثق إستثنائياً إلى خلايا البطانة الداخلية أو القشرة الخارجية.

يوجد العديد من الدراسات المشجعة التى تبين أن الخلايا الجذعية المشتقة من نخاع العظام لها دور فى إلتئام العديد من الأنسجة.

الهدف من الدراسة: دراسة دور العلاج بالخلايا الجذعية فى علاج ثقبوط طبلة الأذن وذلك عن طريق منظار الأذن والفحص الميكروسكوبى والتقييم بإختبارات السمعيات.

المرضى وطرق البحث:

المرضى: تم إجراء هذه الدراسة على ثلاثين مريضاً بمستشفيات الزهراء الجامعى ومستشفى شبين الكوم التعليمى فى الفترة الزمنية من أبريل ٢٠١٤ حتى ديسمبر ٢٠١٨.

مواصفات المرضى:

- السن أكبر من ١٢ سنة.
- موافقة المريض على إجراء البحث.
- الثقب مركزي وجاف أكثر من شهر.
- لم يتم إجراء عملية سابقة على نفس الأذن.

مواصفات المرضى المستبعدون من الدراسة:

- الأطفال الأقل من ١٠ سنوات.
- المرضى الراضين لإجراء البحث.
- مرضى البول السكرى وضعاف المناعة.

طرق البحث: كل المرضى موضع الدراسة تم تعريضهم إلى:

- ١- أخذ تاريخ مرضى كامل مع التركيز على بداية وتطور الحالة وفترة المرض وسبب ثقب طبلة الأذن والعلاج السابق.
- ٢- فحص الأنف والأذنين والحنجرة بدقة مع التركيز على فحص الأذنين بالمنظار بدقة.
- ٣- عمل إختبارات السمع لكل المرضى.
- ٤- عمل فحص طبي شامل مع التركيز على مرضى الجهاز التنفسي العلوى ومرضى البؤر الصديدية المزمنة.
- ٥- سحب عينة من نخاع العظام من الشوكة الأليبية الأمامية من كل مريض.
- ٦- سوف يتم فصل الخلايا أحادية النواة وتقسيمها إلى طبقات طبقاً للكثافة وسوف يتم زرعها فى نسيج هيبيز فى درجة حرارة ٣٧ درجة مئوية فى حضانة ثانى أكسيد الكربون.
- ٧- بعد ٣٦ إلى ٤٨ سوف يتم فصل الخلايا المتلاصقة باستخدام تريسين وكولاجيناز.
- ٨- تم إعطاء الخلايا الجذعية مخففة بمحلول ملح بتركيز ١.٢ لكل مليلتر للمرضى موضعياً على ثقوب الأذن.
- ٩- تم متابعة الحالات عن طريق دراسة قصيرة الأمد (ثلاث مرات أسبوعياً لمدة أسبوعين) وخطة طويلة الأمد (مرتين أسبوعياً لمدة شهر وبعد ستة أشهر) .

ملخص النتائج والتوصيات:

- أغلب الحالات كانوا سيدات بنسبة ٦٦.٧٪ مقابل ٣٣.٣٪ كانوا رجال نظراً لأهمال علاج المرأة فى الريف المصرى.
- أغلب الثقوب كانت مركزية بنسبة ٩٦.٧٪ مقابل ٣.٣٪ للثقوب العلوية.
- نسبة نجاح إلتئام الأذن بعد ٣ شهور ٩٦.٧٪ مقابل ٣.٣٪ أى حالة واحدة فشل إلتئامها.
- أغلب الحالات فى المنطقة العمرية ١٢ إلى ٥٠ سنة بمتوسط ٣١.٩ سنة.
- أغلب الثقوب كانت فى الأذن اليسرى بنسبة ٦٠٪ إلى ٤٠٪ بالناحية اليمنى وذلك حتى يكون الثقب مناسب للجراح الذى يعمل باليد اليمنى.
- نسبة رضا الطبيب كانت ٩٦.٧٪ ونسبة رضا المريض كانت ٩٤.١٪.
- أغلب الحالات كان الثقب صغير ٢ × ٢ مم أو أقل بنسبة ٦٣.٧٪ مقابل ٣٣.٣٪.
- نسبة تحسن وغلق الفرق بين السمع عن طريق الهواء والسمع عن طريق العظام عالية جداً ٩٦.٧٪.
- نسبة تحسن حجم الأذن الخارجية عالية جداً وأيضاً مقياس السمع وضغط الأذن الوسطى.

لذلك ينصح بعلاج الثقوب المركزية الجافة بحجم حد أقصى ٤ × ٤ مم بالعلاج عن طريق الخلايا الجذعية تحت مخدر موضعى بشرط موافقة مكتوبة مسبقاً من المريض على ألا يكون مريض سكرى أو ضعيف المناعة.