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Research Article

Topical Application of Epidermal Growth Factor (EGF) to Changes in Tympanic Membrane Perforation Diameter

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Abstract

Background and Objective: Some researches have already been conducted about the effect of topical application of Epidermal Growth Factor (EGF) in the use of amniotic graft to changes in tympanic membrane perforation diameter. The aim of this research was to know the effects of topical Epidermal Growth Factor (EGF) application in graft amnion usage on perforation membrane's diameter which compared to the control. **Materials and Methods:** The research method used was experimental with randomized pre-test and post-test control group design. Patients with chronic tympanic membrane perforation without infection were injured at the perforated edge using a sickle knife which was then smeared with a topical epidermal growth factor then perforation closes with the amniotic membrane. There were 20 samples consisting of 10 samples with amnion membrane without topical EGF and 10 samples with amnion membrane plus topical EGF. **Results:** The results obtained in the EGF group had significantly reduced perforated diameter ($p < 0.05$) by 21.1% during the 2nd week; whereas in the group without EGF has not been reduced at all. This means that there was a faster decrease in perforated diameter in the topical group treated with EGF than in the top group without the topical EGF, since the 2nd week and significantly different in the 5th week. **Conclusion:** Epidermal growth factor plays a role in shrinking the perforation diameter of the tympanic membrane on the use of amniotic membranes as graft.

Key words: Tympanic membrane, perforation diameter, topical group, amnion membrane, epidermal growth factor

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tympanic membrane perforation or leak in the eardrum is a frequent occurrence¹. Perforation in the tympanic membrane may be due to trauma or middle ear infections and can usually close spontaneously unless a large perforation or chronic infection in the middle ear will perforate permanently. It is a hole in the tympanic membrane that cannot close spontaneously within 3 months after perforation².

Estimated prevalence of tympanic membrane perforation by about 1-3% in the population in the United States³. The prevalence of tympanic membrane perforation due to OMSK in developing countries ranges from 5-10%, whereas in developed countries 0.5-2%. It is estimated that around 10 million Indonesians suffer from OMSK. In Dr. Wahidin Sudirohusodo Makassar Hospital, the number of cases of OMSK during the period 2012-2016 is as many^{4,5} as 1,394.

Perforation of a permanent/chronic tympanic membrane may cause various morbidities including hearing impairment, chronic ear discharge, recurrent middle ear infections, cholesteatomas and intracranial infections via perforation route. Thus it should be done therapy to close perforation of the tympanic membrane. Closure of tympanic membrane perforation can be performed by operative and conservative^{6,7}.

Conservative techniques reduce costs, minimal manipulation, relatively simple operating procedures, reduce length of operation, reduce costs can be done simultaneously on both ears, reduce the risk of surgery and can be done outpatient^{8,9}. Efforting closure of perforated permanent tympanic membrane conservatively has long been tried by means of all edges of permanent tympanic membrane perforation created new wounds. The use of caustic material was first introduced by Roosa in 1867 using silver nitrate to create new wound perforated edge and trichloroacetate was first used in 1895. Then a caustic combination with paper as a bridge gave better results. Based on this Derlacki popularized the paper patch technique^{10,11}. This conservative method takes a long time, the average closing time of 7 months 4 days, while the new wound production should be repeated every 2 weeks and on paper cover euted two times a day euthymol solution as much as two drops. Success rate reached 45% of 131 perforated permanent tympanic membrane⁷.

Since the artificial concept of tympanic membrane was introduced, various surgical techniques and graft materials were used to improve the healing of tympanic membrane perforations^{12,13}. Graft to close perforation of the tympanic membrane using body tissue taken from elsewhere (perichondrium, fascia, adipose fat, etc.)¹⁴. Currently, the use

of amnion membranes to close perforation of the tympanic membrane has been widely reported with positive results. A study reported satisfactory results on the use of amnion membranes against 14 cases. After 6 weeks postoperative, 8 cases (57.1%) had decreased perforation size and 6 cases (42.9%) had complete MT perforation closure. The mean water bone gap prior to operation decreased from 23.0 ± 10.1 to 16.8 ± 7.4 db after surgery¹⁵.

There are several bioactive molecules or growth factors that have been used in previous studies, related to healing of MT perforations. Bioactive molecules that are currently being studied are the epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and hyaluronic acid (HA). Function of growth factors in general to stimulate wound healing on the tissues. This biomolecule can be obtained anywhere with a relatively cheaper price. Research, compared the effects of EGF with bFGF on healing of large MT perforations, showed significantly different results. A study of 86 patients for 3 months showed that perforated MT cover in EGF, bFGF and control group were 74 cases (86.2%), 77 cases (89.3%) and 62 cases (72.4%), respectively. The time required for perforation closure was significantly longer in the control group ($p < 0.01$) than in the EGF and bFGF groups. While the perforation closure time between EGF group and bFGF did not differ significantly ($p = 0.82$)^{16,17}. While a study used autologous serum ear drops as a regulator factor, amnion as a bridge and the use of silver nitrate at the perforation edge to create new wounds. Autologous serum has a growth accelerator of epidermal growth factor (EGF), transforming growth factor $\beta 1$ (TGF- $\beta 1$) and fibronectin, the result of tympanic membrane perforation closure occurs in 11 ears of 19 treated ears².

Some actual publications have already been conducted which show that this research has started from *in vitro* experiments to simple clinical trials with few samples and clinical trials with large samples, this study would like to support existing studies with different samples, namely in Makassar, South Sulawesi, Indonesia and with different duration of time with has been published previously. The objective of the study was to determine the effect of topical application of Epidermal Growth Factor (EGF) in the use of amniotic graft to changes in tympanic membrane perforation diameter.

MATERIALS AND METHODS

This was an experimental research with the randomized pre-test and post-test control group design approaches.

Research location: The study was conducted in ENT Clinic Dr. Wahidin Sudirohusodo Hospital in June-October, 2017.

Population and samples: The population of this research were all of the patients who got the treatments on ENT Clinic Dr. Wahidin Sudirohusodo Hospital with the one or more complaints on their ears, such as, decreasing on hearing, watery ears and buzzing ears. In the period of June-October, 2017, there were 20 samples from 10 patients who got the treatment and 10 patients as a control. Inclusion criteria were the patient with perforation in dry tympanic membrane, unhyperemic and suppurative chronic inactive otitis media, patients with persistent perforation tympanic membrane (3 months), patient post tympanoplasty operation who failed to fix for 3 months, perforation central and small to medium size (<5 mm) did not use the anti-EGF medicines for a month, accept for being the part of research samples. The exclusion criteria were the patients who rejected the involvement on research had suffered active suppurative chronic otitis media with kolestetoma and severe allergies.

On the execution of the research, every treatment was conducted with the permission of the patients by informed consent sheet and eligible all ethics requirements from ethics commission of bio-medic on human, Medical Faculty of Hasanuddin University.

Procedures: Anamnesis treatment were given to the samples, after that the samples then got the ENT physical examination such as, otoscopy, anterior rhinoscopy, posterior rhinoscopy and faringcopy.

Perforation timpani membrane was measured with prepared sonde and got myringoplasty treatments. The patient was sat in examination chair and the examiner was on the ears side of the treated patient. Patient was prepared with cleaning procedures on external ear canal and disinfected from external ear canal up to earlobe with 70% liquid alcohol. After that, they got the local anesthesia treatment using a small piece of cotton which contains 10% Lidocaine hydrochloride anhydricum (Xylocaine Astra Zeneca, USA) and

located in the surface of MT through external ear canal. Telescope was used in the next step on the perforation edge and getting stripped by sickle knife to make a new slice on its surface. On the first group, topical GF was smeared on the perforation edge which was sliced around 0.1 mL, after that, closed the perforation using amnion membrane which already shaped. On the second group, the edges of sliced perforation were closed with the amnion membrane which already shaped. If the perforations are convinced to be closed by the amnion membrane, then external ear canal could be closed with dry sterile ears pack, make sure that the ears pack did not touch amnion membrane (advisable to locate on the cartilage pars). The patients were asked to come every 5 days for getting an examination to know the development of perforation diameter changes process and advised to not get the ear wet, not doing Valsalva, not to eat food that can cause inflammation on nose and throat. Before the patient leaves, they were provided with medicines: Antibiotics, antihistamine, decongestant. The grafts were evaluated for 4 weeks or until the perforation diameter changes on timpani membrane are observable.

Data analysis: Collected data were grouped based on the kinds of data and were chosen for the appropriate statistical method and analyzed with statistic SPSS program for windows. The probability value was considered significant when the value was less than 0.05.

RESULTS

The research was conducted in Wahidin Sudirohusodo Hospital since June-October, 2017. There were 20 samples total which fulfilled all of the inclusion requirement. It consists of 10 samples with amnion membrane cover without EGF and 10 samples with amnion membrane cover with EGF. Treatment grouping was randomized. The result of homogeneity sample test on both groups based on the samples characteristic which presents in Table 1.

Table 1: Characteristic patients based on group

Characteristics	Groups		p-value
	Membrane without EGF (n = 10)	Membrane with EGF (n = 10)	
Age (years)	33.5 ± 13.3	34.2 ± 13.3	0.908**
Sex (M/F)	3/7	6/4	0.370*
Perforated area (K/S)	2/8	2/8	1.000*
Perforation location (AI/PI)	6/4	8/2	0.628*
Diameter of perforation (mm)	2.65 ± 0.63	2.80 ± 0.63	0.529***

*Fisher's exact test, **Independent sample t-test, ***Mann whitney U test

Table 2: Comparison of diameter on perforation timpani membrane for both of group in different observation time

Observation time	Membrane without EGF		Membrane with EGF		*p-value
	Diameter (mm)		Diameter (mm)		
	Min/max (median)	Mean±SD	Min/max (median)	Mean±SD	
Before	2.5/4.0 (2.50)	2.65±0.63	2.5/4.0 (3.00)	2.80±0.63	0.473
1 week	2.5/4.0 (2.50)	2.63±0.63	2.5/4.0 (3.00)	2.80±0.63	0.473
2 weeks	2.5/4.0 (2.50)	2.65±0.63	1.5/4.0 (2.25)	2.25±0.79	0.124
3 weeks	2.0/4.0 (2.50)	2.69±0.70	1.0/3.5 (2.00)	1.85±0.85	0.064
4 weeks	1.5/3.5 (2.00)	1.70±0.65	0.5/3.0 (1.75)	1.50±0.78	0.083
5 weeks	1.0/3.5 (2.00)	2.05±0.72	0.0/2.5 (1.00)	1.05±0.83	0.015

*Mann Whitney U test, EGF: Epidermal growth factor

Table 3: Number of reduction perforation diameter based on observation time on each group

Groups	Observation time (weeks)	Diameter changes (mm)				*p-value
		Before	After	Reduction	Coverage (%)	
Membrane without EGF	2	2.65±0.63	2.65±0.63	0.00±0.00	0.0	1.000
	3	2.65±0.63	2.69±0.70	0.10±0.21	4.0	0.157
	4	2.65±0.63	1.70±0.65	0.55±0.16	21.7	0.002
	5	2.65±0.63	2.05±0.72	0.60±0.21	20.0	<0.001
Membrane with EGF	2	2.80±0.63	2.25±0.79	0.55±0.28	21.1	0.005
	3	2.80±0.63	1.85±0.85	0.95±0.50	35.8	0.005
	4	2.80±0.63	1.50±0.78	1.30±0.48	48.3	0.004
	5	2.80±0.63	1.05±0.83	1.75±0.49	60.0	<0.001

*Wilcoxon test, EGF: Epidermal growth factor

It was cleared that statistical analysis on Table 1, it showed that there was no significant difference ($p>0.05$) between two group based on characteristics of age, sex, perforated area before surgery, perforated location and diameter of perforation before treatment. It means that both of group could be concluded as a homogeny, based on those characteristics or not significantly influence the result on both groups.

Based on the perforation diameter comparison analysis between both of group on each observation time, it can be concluded that there is no significant diameter difference ($p<0.05$) before the treatment up to 2 weeks after the treatment, even though there is tendency of perforation diameter are smaller on the group membrane with EGF (2.25 ± 0.79 vs. 2.65 ± 0.63). On the 3rd weeks after the treatment, perforation diameter on the membrane with EGF group becomes smaller and different compared by the group without EGF (1.85 ± 0.85 vs. 2.69 ± 0.70 mm) even though there is no different statistically ($p>0.05$). Data showed similar result between the 3rd week and 4th week, at the 4th week, perforation diameter on the membrane with EGF group became smaller and different compared by the group without EGF (1.70 ± 0.65 vs. 1.50 ± 0.78 mm) even though there was no different statistically ($p>0.05$). On the 5th week, there was a significant difference ($p<0.05$) between both of group, the group with EGF were smaller compared by the group without EGF (1.05 ± 0.83 vs. 2.05 ± 0.72 mm). It means

that there was a decreasing on diameter size which was faster on the EGF group compared by the non-EGF group, it happened in the 2nd week and getting more significant on the 5th week. In term of observing this change, the analysis was continued to compare diameter changes on every observation time and to calculate a percentage of perforation coverage on the tympanic membrane after the treatment (Table 2).

In Table 3 can be seen clearly that in the group EGF. perforation/coverage perforation has already significantly decreased ($p<0.05$) up to 21.1% in the 2nd week; while on the group without EGF still stagnant without decreasing phenomena. On the 3rd and 4th week the coverage respectively 35.8; 48.3 and 60% on EGF group. Meanwhile on the group without EGF respectively 4.0; 21.7 and 20.0%. It means that the perforation coverages were faster on the EGF group. To convince this coverage result, the analysis was continued with Mann-Whitney test for comparing percentage of that perforation coverage in tympanic membrane between those two groups.

Table 4 indicated that there was a significant percentage of closure percentage ($p<0.05$) between the EGF group and without EGF after 2 weeks of action up to 5 weeks after the action. Closure of perforation was significantly faster in the group given EGF compared with no EGF or in other words EGF administration accelerated the closure of tympanic membrane perforation.

Table 4: Comparison of changes in diameter of the tympanic membrane perforation between the two groups at various observation time

Observation time (weeks)	Membrane without EGF		Membrane with EGF		*p-value
	Diameter changing (mm)		Diameter changing (mm)		
	Min/max (median)	Median closes (%)	Min/max (median)	Median closes (%)	
2	0.0/1.0 (0.50)	0.0	0.5/1.0 (0.50)	21.1	<0.001
3	0.0/0.5 (0.00)	4.0	0.5/2.0 (1.00)	35.8	<0.001
4	0.5/1.0 (0.50)	21.7	1.0/2.5 (1.00)	48.3	<0.001
5	0.5/1.0 (0.50)	20.0	1.5/3.0 (1.50)	60.0	<0.001

*Mann-whitney U test, EGF: Epidermal growth factor

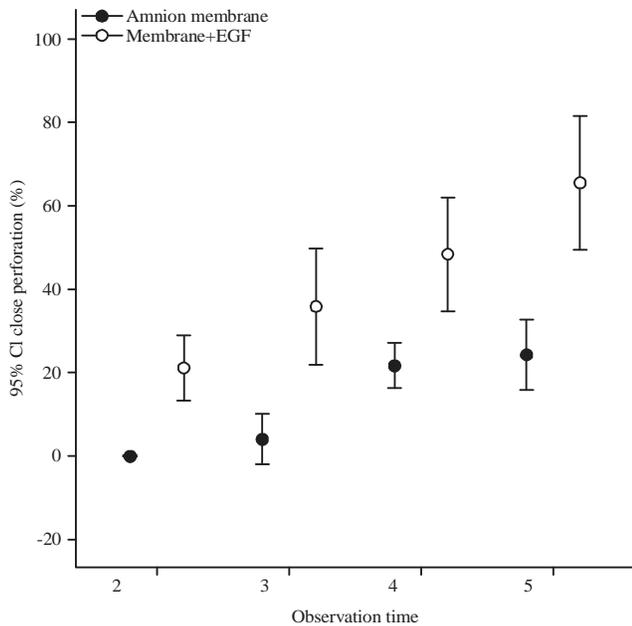


Fig. 1: Percentage of tympanic membrane perforation closure in both groups and various observation times after treatment

Based on the graph shown in Fig. 1, the percentage graph of closure perforation of the tympanic membrane was greater in both groups based on the duration of time after the action. and the percentage of closure of the tympanic membrane perforation was always higher in the EGF group from the 2nd week until the 5th week.

DISCUSSION

All patients were followed up for size transformation of perforated diameter on day 5, 2 weeks, 3 weeks and 1 month. The results obtained in the group given topical EGF size of the tympanic membrane perforation became smaller than the original size starting in the second week. Whereas in the group without topical EGF obtained diminution in size of tympanic membrane perforation on the average starting at the 3rd week.

Manipulation of cells bridges and regulatory factors were needed to stimulate the occurrence of tissue regeneration. In this study, sickle knife was used to make a new wound using in cell manipulation. Beside that, amnion membrane as bridge / bridge and epidermal growth factor as regulation factor¹⁸.

The use of amnion membranes to close perforation of the tympanic membrane had been widely reported with positive results. The non-viable amnion membrane was easily found and the price was relatively cheap. Moreover can be stored for a long time⁷. The selection of amnion membranes as a bridge based on several considerations that epithelial tissue and amniotic stroma contain growth factors as epithelial facilitators. Namely epidermal growth factor (EGF). Transforming growth factor α (TGF- α), keratinocyte growth factor (KGF) and basic fibroblast growth factor (bFGF) transforming growth factor β 1-2 (TGF- β 1-2). The amniotic stroma contained the components of type IV collagen lamin in integrins 6 and 4 which were components of the extracellular matrix as the constituent material of the epithelial basement membrane to accelerate the epithelialization process. Amniotic membranes that had been preserved for one month still contain these growth factors. As evidenced by Polymerase Chain Reaction (PCR) and ELISA examination methods¹⁹.

Several factors have to be considered in successful closure of perforation of permanent tympanic membrane. Growth factors that trigger the growth of new blood vessels have the potential to accelerate the healing of connective tissue injuries. There were four kinds of angiogenic growth factors. Namely fibroblast growth factor (FGF) transforming growth beta factor (TGF- β). Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF)¹⁸. Chauvin required perforation of the tympanic membrane used only growth factors wherein it was found the transformation in perforation closure occurred on days 21-23 in marmot animal studies²⁰. In this study used amnionic membranes as bridges and topical growth factor there was faster transformation in closure of tympanic membrane perforation in the 2nd week (day 10-14)^{18,20}.

A study about perforation of chronic tympanic membrane have been experimented on chinchilla animals. However, the results reported in topical EGF used in human tympanic membranes did not show the desired effect as in animal experiments. This study did not make new sore on the edge of perforation. It was concluded that the closure of perforation of the tympanic membrane was not determined by EGF concentration. Perhaps making a new sore on the perforated edge improved the results²¹. In this study, in addition to topical epidermal growth factor and the use of amniotic membranes as a graft/bridge on the edge of the tympanic membrane the perforation was made of new sore with the result that the perforation size change becomes smaller.

CONCLUSION

Epidermal growth factor plays a role in shrinking the perforation diameter of the tympanic membrane on the use of amniotic membranes as graft.

SIGNIFICANCE STATEMENTS

This study provides an explanation and supporting the previous research about epidermal growth factor plays a role in shrinking the perforation diameter of the tympanic membrane on the use of amniotic membranes as graft.

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