Arlex Michael O. Atanacio, MD
Emily Grace Teodoro-Estaris, MD

Department of Otolaryngology-Head and Neck Surgery
Baguio General Hospital and Medical Center

Epidermal Growth Factor Application versus Observation on Healing of Acute Tympanic Membrane Perforations: A Randomized Open Label Clinical Trial

ABSTRACT

Objective: To compare the effect of topical epidermal growth factor (EGF) instillation versus observation alone on healing of acute tympanic membrane perforations in terms of closure and hearing test results.

Methods:

Design: Randomized, Open label, Clinical Trial
Setting: Tertiary Government Training Hospital
Participants: Seventeen (17) ENT-HNS OPD patients aged between 18 to 65 years old diagnosed with acute tympanic membrane perforation were included in the study. Group A underwent observation while group B was treated with recombinant human EGF solution. Follow-up was on a weekly basis (7th, 14th, 21st and 28th days) where video otoscopy for documentation and measurement of perforation using ImageJ™ software was done. Pure tone audiometry was used to compare hearing improvement pre and post study in both observation and treatment groups.

Results: At baseline, there was no significant difference in the sizes of perforations: 24.20 ± 9.95 (treatment) vs. 32.64 ± 11.62 (observation) with a p-value of .131. Following treatment, mean changes in perforation size were significantly greater in the treatment group compared to the observation group from baseline to day 7 (M = -9.08, n = 15.11 vs. M = -1.06, n = 31.58); p = .009; day 7 to 14 (M = -6.37, n = 13.78 vs. M = -0.79, n = 30.79); p = .003; and from day 14 to 21 (M = -5.65, n = 10.89 vs. M = -0.72, n = 30.07); p = .004 but not from day 21 to 28 (M = -4.16, n = 13.99 vs. M = -0.36, n = 29.71; p = .021. From baseline pure tone averages, four participants with mild hearing loss and two with moderate hearing loss achieved normal hearing in the treatment group (while one each with moderate and severe hearing loss did not improve). None of the observation group participants had improved hearing.

Conclusion: Based on our limited experience, topical EGF can be used for traumatic tympanic membrane perforation and otitis media with dry ear perforation during the acute phase or within 3 months of perforation.

Keywords: *tympanic membrane perforation; ear trauma; otitis media; hearing impairment; human epidermal growth factor; tympanic membrane rupture; wound healing*

The Baguio General Hospital and Medical Center (BGHMC) had a total of 309 tympanic membrane (TM) perforations in 2017, 164 in 2018, and 156 from January to July 2019 alone.[1] Based on our average data, a 10-year projection for patients with chronically perforated TM and hearing loss may reach 2,880. This condition may result from previous infection, traumatic

Correspondence: Dr. Emily Grace Teodoro-Estaris
Department of Otolaryngology-Head and Neck Surgery
Baguio General Hospital and Medical Center
Upper Governor Pack Road, Baguio City 2600
Philippines
Phone: +63 74 244 5042
Fax: +63 74 442 3809
Email: emdestaris@yahoo.com

The authors declared that this represents original material that is not being considered for publication or has not been published or accepted for publication elsewhere in full or in part, in print or electronic media; that the requirements for authorship have been met by all the authors, and that each author believes that the manuscript represents honest work.

Disclosures: The authors signed a disclosure that there are no financial or other (including personal) relationships, intellectual passion, political or religious beliefs, and institutional affiliations that might lead to a conflict of interest.

Presented at Baguio General Hospital Annual Research Contest 2019 Podium Presentation (1st Place). BGHMC Cancer Center Conference Hall, Baguio City. December 4, 2019.

manipulation, and barotrauma like diving or through air travel.[2] Moreover, persistent allergies, upper respiratory tract infection or presence of a suppurative ear discharge may prevent healing within 3 months. Perforations of the TM may present with active infection or a dry ear perforation. Based on location, they are classified as either central or peripheral; and in terms of duration, are considered acute when present for less than 3 months and chronic when present more than 3 months.[3]

Management in our institution consists of instilling antibiotic otic drops, treating co-morbid conditions, and achieving a safe dry ear, and a 'watch and wait' policy is the practiced standard of care for dry TM perforations. Patients who fail to attain full closure within the 3-month observation period are advised paper patch and fat graft tympanoplasty, but the success rates for these procedures vary. Moreover, data on follow-up for progress of healing, transition to chronicity, and hearing status is limited.

Recombinant human epidermal growth factor (EGF) is a potent stimulator of epithelial and endothelial cells and fibroblast proliferation which has potential as healing agent that has been used in acute and chronic TM perforations.[4-6] Various clinical and experimental studies have suggested that topical application of EGF improves closure rate and shortens closure time in TM perforations, with closure rates ranging from 71.42% to 97.8%[7-8] and no significant side effects reported.[9]

With the intention of exploring the use of EGF on TM perforations in our practice, this study aimed to compare the effect of topical epidermal growth factor instillation versus observation alone on healing of acute tympanic membrane perforations in terms of closure and hearing test results. We hypothesize that the topical application of EGF on acute tympanic membrane perforation would accelerate healing and closure compared to observation.

METHODS

With Baguio General Hospital and Medical Center (BGHMC) Research Ethics Committee approval (BGHMC-REC-2019-31), this study used a randomized open label clinical trial design.

Adult patients aged 18 to 65 years who were diagnosed with acute tympanic membrane perforation at the BGHMC Otorhinolaryngology - Head and Neck Surgery (ORL-HNS) outpatient department (OPD) from August to September 2019 were serially considered for inclusion in the study. Inclusion criteria were ≤ 50% perforated tympanic membrane (TM). Excluded were patients with active ear infection, persistently untreated allergic rhinitis, those who underwent otologic surgical management, females who were pregnant or who planned to conceive for the entire duration of the study. Informed consent was obtained from prospective participants meeting inclusion and exclusion criteria.

The sample size was computed at 95% confidence interval, 80% power, 5% margin of error, and 81% exposed with outcome using OpenEpi version 3.01 (Open-Source Epidemiologic Statistics for Public Health, *www.OpenEpi.com*, updated 2013/04/06). The total sample size was computed at 16. Due to the varying range of efficacy for Epidermal Growth Factor (EGF), the sample size computation was based on the initial study done by Amoils[10] where the exposure outcome was based on the lowest possible successful treatment using topically applied EGF of 81% without manipulation of the perforated tympanic membrane.

One researcher (AMOA) provided an audio-visual presentation of the materials, drug, procedure, and table of follow-up schedules in a manner or language that the prospective participants understood. A directed clinical history was obtained, and thorough physical examination was performed, including basic otoscopy and tuning fork testing using 512 Hz, with information on the causes of the perforation collected. Pure tone audiometry was performed by a 2nd year ORL-HNS resident using an Interacoustics Diagnostic Audiometer, (AD226, Interacoustics A/S Audiometer Allé 1 5500 Middelfart, Denmark).

Randomization was made via coin flip, heads for the control/observation group (A), tails for the treatment group (B). Those randomized to group A were instructed to come for weekly follow-ups and documentation or as the need arose (e.g., when there was an upper respiratory tract infection, immersion in water or ear discharge). Those randomized to group B were treated with EGF (as described below) and were advised to follow up on a weekly basis for evaluation or when there were concerns.

The tympanic membrane was photographed using a digital video camera (Medone Innotech, LL 250M, CCU 1500 with camera, Gyeonggi-do, Korea) attached to a 4mm x 100mm rigid 0^0 endoscope (Tianjian Bolang Science-Technology Development Co., Ltd, 2018, Tian Jin Shi, China), the perforation size was recorded using ImageJ™ version 1.52 software (Wayne Rasband, US National Institutes of Health *https://imagej.nih.gov/*) where the equation generated was as follows:

$$\frac{P}{T} \; x\ 100 \text{ percent} = \text{percentage perforation}$$

Where: P is the area (in pixels^2) of the tympanic membrane perforation
T is the total area (in pixels^2) of the tympanic membrane

The size, site and adequate margins of the perforated tympanic membrane and external auditory canal were documented. After performing physical examination, leaflets on proper ear care were given to each participant with the proper advice on how to maintain a dry ear. Attached to the leaflet were follow-up schedules and the contact person for any concerns. Evaluation of perforation and application of EGF was carried out by a BGHMC ORL-HNS graduate, a Medical Officer IV.

After screening for any history of adverse drug reactions, an initial topical application of 3 drops of the test drug on the antecubital area with a 1 x 1 cm diameter surrounded by a black surgical hypoallergenic pen skin marking with the date and time was performed. A photograph of the topical skin application and skin marking was obtained, with waiting time of 30-60 minutes to note for any adverse reaction.

For the EGF (treatment) group, each participant was asked to lie down on an OPD bed with the affected ear superior (lateral recumbent). The external auditory canal was cleaned using a cotton pledget soaked with povidone-iodine. The affected ear received treatment of 0.4ml (5 drops) of recombinant human EGF solution (rhEGF; 0.20mg/200mcg; Easyef, Daewoong Pharmaceuticals, Philippines), and the position was

maintained for 30 minutes to allow adequate contact of the drug and the tympanic membrane. Patients were then asked to sit up at the side of the examination bed and monitored for any untoward reactions for 30-60 minutes. The control group on the other hand was advised to keep their ears dry, monitor for any discharge and come for regular follow-up.

Measurement of the percentage of closure was performed weekly for both groups. Those who failed to attain full tympanic membrane closure after the 28th day of monitoring were asked to continue follow-up every 14 days thereafter. Pure tone audiometry was repeated at the fourth week by a 2nd year ORL-HNS resident using the same audiometer.

Data analysis

Descriptive and inferential statistics were utilized for the description and analysis of data, respectively. Data was encoded using Microsoft Excel Office Professional Plus 2019 (Microsoft Corp., Redmond WA, USA). Descriptive statistics made use of means and was used in determining the average differences in perforation size of both the control and treatment group. Also, frequencies and percentages were used in the pre and post PTA of the patients. Test of normality was conducted using the Kolmogorov-Smirnov and Shapiro-Wilk tests to determine the appropriate test statistics that would be applied. Wilcoxon Signed-Ranks test was used to determine whether the differences between pre and post treatment observations were significant. A p-value of ≤ .05 was considered significant.

RESULTS

Out of 24 potential participants who met inclusion and exclusion criteria and were initially considered for this study, a total of 17 participants completed it. There were 6 males and 11 females, with ages ranging from 18 to 65 years old. Eight were randomized to the treatment group (4 males, 4 females) and nine to the observation group (3 males, 6 females). The mean ages for the treatment and observation groups were 45.5 (SD: 9.12) and 36.7 (SD 14.26), respectively.

There was only one TM perforation per participant, eight (8) left ear perforations and nine (9) right ear perforations. All perforations for both groups were central. Baseline sizes for perforations in the treatment group were 14, 34, 31, 19, 38, 10, 20 and 24 %. *(Figure 1)* For the observation group, perforation sizes were 38, 41, 35, 41, 29, 13, 35, 13 and 45 %. *(Figure 2)* The treatment group had four (4) traumatic membrane perforations and four (4) otitis media with dry ear perforations. The control group had four (4) traumatic ear perforations and five (5) otitis media with dry ear perforations. *Figures 1* and *2* show hte serial tympanic membrane photographs for the treatment and observation groups, respectively.

Table 1 compares the observation and treatment groups in terms of mean sizes of perforation on initial evaluation (baseline), and days 7, 14, 21, and 28. At baseline, there was no significant difference in the sizes of perforations: 24.20 ± 9.95 (treatment) vs. 32.64 ± 11.62 (observation) with a p-value of .131.

In both observation and treatment groups, there was a significant decrease in mean perforation size from baseline on day 7, 14, 21, and 28. However, comparison from paired periods showed no significant

Table 1. Mean perforation size for observation and treatment groups: baseline, day 7, 14, 21 and 28

	Group	Mean	Std. Deviation	p-value
Baseline	Observation	32.64	11.625	.131
	Treatment	24.20	9.950	
Day 7	Observation	31.58	11.753	.014
	Treatment	15.11	12.657	
Day 14	Observation	30.79	12.284	.017
	Treatment	13.78	11.032	
Day 21	Observation	30.07	11.726	.011
	Treatment	10.89	10.696	
Day 28	Observation	29.71	11.803	.007
	Treatment	13.99	4.245	

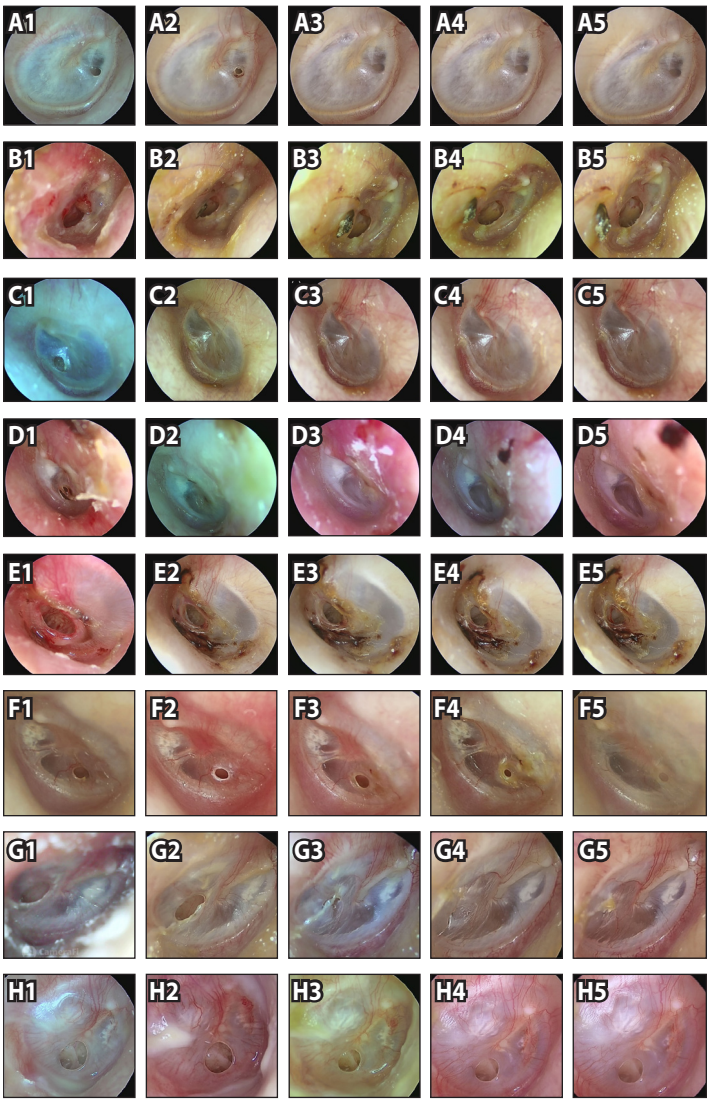

Figure 1. Treatment group serial tympanic membrane photos, top to bottom, patients A to H (rows). Left to right, numbered 1 (baseline), 2 (day 7), 3 (day 14), 4 (day 21), and 5 (day 28). For example, B3 is a tympanic membrane photo of patient B on day 14.

decrease in perforation size from one period to the next for the observation group, while there was a significant decrease in perforation size from day 7 to 14 and from day 14 to 21 in the treatment group, but no significant decrease in perforation size from day 21 to 28.

Table 2 shows a significant decrease in mean perforation size in the observation group from baseline on day 7, 14, 21, and 28.

Table 2. Observation group differences in perforation size from baseline: day 7, 14, 21 and 28

Period	Mean Difference	Std. Deviation of Differences	p-value
Day 7	1.063	1.232	.032
Day 14	1.854	1.687	.011
Day 21	2.570	1.413	.001
Day 28	2.929	2.225	.004

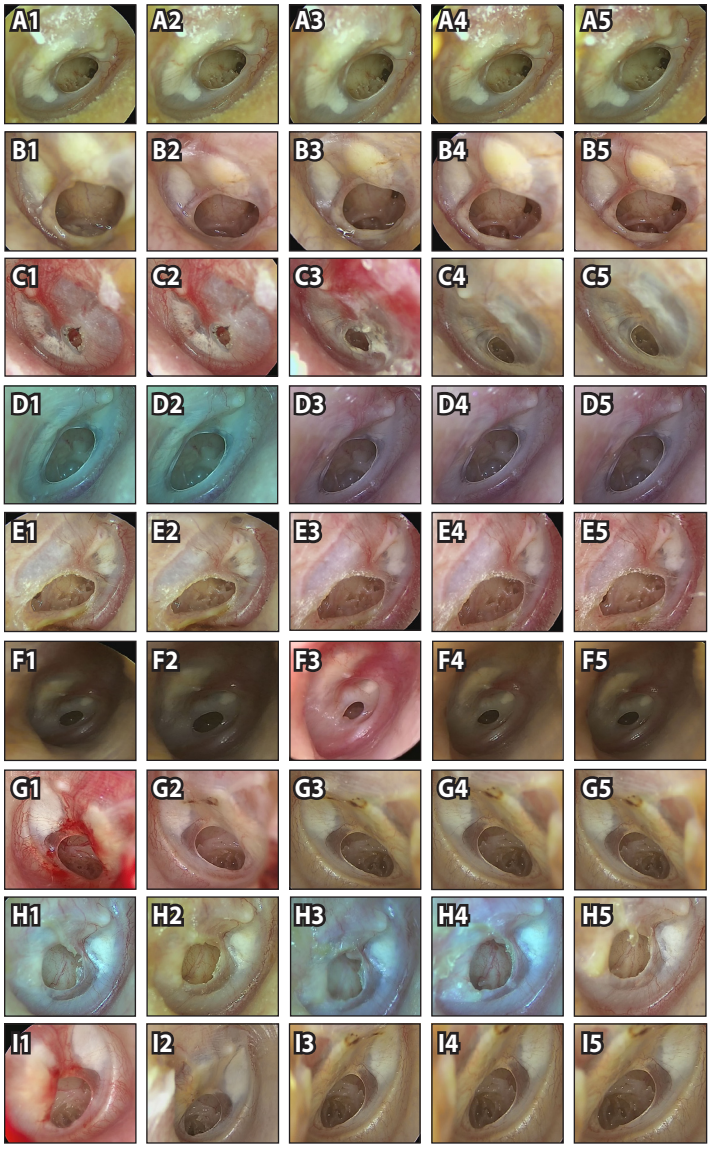

Figure 2. Observation group seriel tympanic membrane photos, top to bottom, patients A to I (rows). Left to right, numberecd 1 (baseline), 2 (day 7), 3 (day 14), 4 (day 21), and 5 (day 28). For example, C4 is a tympanic membrane photo of patient C on day 21.

However, *Table 3* shows no significant decrease in perforation size from one period to the next for the observation group on paired period comparisons.

Table 3. Observation group differences of size perforation from paired periods

Paired Periods	Mean Difference	Std. Deviation of Differences	p-value
Day 7 and 14	0.791	1.255	.095
Day 14 and 21	0.716	0.955	.055
Day 21 and 28	0.359	1.216	.402

Table 4 shows a significant decrease in the mean size of perforation from baseline on day 7, 14, 21 and 28 in the treatment group.

Table 4. Treatment group differences in perforation size from baseline: day 7, 14, 21 and 28

Period	Mean Difference	Std. Deviation of Differences	p-value
Day 7	9.083	10.890	.050
Day 14	10.020	2.810	.000
Day 21	14.802	4.274	.001
Day 28	18.480	5.324	.027

Table 5 shows significant decreases in perforation size from day 7 to 14 and from day 14 to 21, but not from day 21 to 28 on perforation size comparisons from paired periods for the treatment group.

Table 5. Treatment group differences of size perforation from paired periods

Paired Periods	Mean Difference	Std. Deviation of Differences	p-value
Day 7 and 14	6.367	2.931	.003
Day 14 and 21	5.652	3.428	.021
Day 21 and 28	4.157	2.860	.128

Wilcoxon signed rank test revealed that mean changes in perforation size were significantly greater in the treatment group compared to the observation group from the initial application of EGF to day 7 of perforation size evaluation (M = -9.08, n = 15.11 vs. M = -1.06, n = 31.58); p = .009; from the 7th to the 14th day of evaluation (M = -6.37, n = 13.78 vs. M = -0.79, n = 30.79); p = .003; and from the 14th to the 21st day of evaluation (M = -5.65, n = 10.89 vs. M = -0.72, n = 30.07); p = .004 but not from the 21st to the 28th day of evaluation (M = -4.16, n = 13.99 vs. M = -0.36, n = 29.71; p = .021 *(Table 6)*

Table 6. Comparative analysis of mean changes in perforation size between treatment group and observation groups

Time Interval	Mean Changes in Perforation Size		Test Statistic	P-value[a]
	Treatment	Observation		
Baseline to day 7	-9.08	-1.06	2.598	.009*
Day 7 to 14	-6.37	-0.79	2.946	.003*
Day 14 to 21	-5.65	-0.72	2.867	.004*
Day 21 to 28	-4.16	-0.36	2.311	.021*

Negative values indicate decrease in the perforation size
aWilcoxon Singed-Ranks test
**significant @ p-value ≤ .05*

Pure tone averages in the treatment group included four (4) mild hearing loss, three (3) moderate hearing loss, and one (1) severe hearing loss. After EGF treatment, six (6) participants had improvement in pure tone average from mild and moderate hearing loss to normal hearing thresholds. Two (2) participants with moderate and severe hearing loss respectively did not have significant improvement in pure tone average. Pure tone averages for the observation group had one (1) normal hearing, one (1) mild hearing loss, six (6) moderate hearing loss, and one (1) severe hearing loss. After observation, no difference in pure tone averages was noted. However, these samples were not adequate to draw inferences from. *(Table 7)*

Table 7. Pre and post pure tone audiometry results of treatment and observation groups

TREATMENT GROUP				
BASELINE PTA	POST PTA			
	Normal	Mild	Moderate	Severe
Normal	-	-	-	-
Mild	4 (100.0)	-	-	-
Moderate	2 (66.7)	-	1 (33.3)	-
Severe	-	-	-	1 (100.0)
OBSERVATION GROUP				
BASELINE PTA	POST PTA			
	Normal	Mild	Moderate	Severe
Normal	1 (100.0)	-	-	-
Mild	-	1 (100.0)	-	-
Moderate	-	-	6 (100.0)	-
Severe	-	-	-	1 (100.0)

DISCUSSION

Healing of the tympanic membrane occurs spontaneously in 88% of traumatic perforations without intervention, as injury around the perforation triggers proliferation of the squamous epithelium along with various growth factors to induce healing.[10]

Our study suggests that EGF seems effective in terms of decreasing the size of the perforation and promoting the growth of epithelial tissue. Overall, the treatment group presented significant epithelial growth in healing acute tympanic membrane perforations.

Our study suggests the same outcome by Lou, *et al.*[6] where patients who received EGF treatment showed accelerated rate of tympanic membrane closure compared to observation alone. In terms of clinical applications,[11] EGF could benefit patients in need of immediate clearance for physical examination such as applicants to the Philippine National Police and Philippine Military Academy as well as those at risk of chronic water exposure such as members of the Navy, swimmers, and chronic users of in-ear or over the ear headphones such as those in the field of telecommunication or Business Process Outsourcing (BPO) companies.

Improvement in hearing corroborated the findings of Lou, *et al.*[6] where morphology of the healed tympanic membrane would not affect the final structure of the TM in terms of sound conduction. As seen in previous studies, the final structure of the healed perforation of the tympanic membrane neared that of the normal tympanic membrane compared to the observation group where there was a deficient fibrous layer of the tympanic membrane.[5,6,10] This could explain the difference between the EGF treatment group and observation group where there was an improvement of hearing in the treatment group as documented by their audiometric results.

This study was limited to the observation of the morphology of the healed perforations since we could not evaluate the histology of human tympanic membranes. [6] Likewise, the observation period was limited to one month. We could extend the follow-up period to at least three months to check for reperforations or increase in the perforation size of those which partially healed[12] and to check also for the development of granulation tissue of the EAC[6] or formation of cholesteatoma in the middle ear. [5] As we limited our study to acute perforations, future studies can investigate the use of EGF in chronic tympanic membrane perforations, where it has shown significant closure rates and hearing gain.[11, 12] The unavailability of EGF is another limitation as it is not readily available in the locality and not listed in the Philippine National Drug Formulary (PNDF). It cannot be prescribed for out-patient use and has to be instilled by a trained physician. Other limitations of the study include the small sample size, and the generalizability of the outcome needs to have a greater number of participants to attain a better comparison of the efficacy of the intervention drug. Double blinding future studies with the use of EGF could also decrease bias.

Meanwhile, based on our limited experience, topical EGF can be used for traumatic tympanic membrane perforation and otitis media with dry ear perforation during the acute phase or within 3 months of the perforation.

REFERENCES

1. Annual statistical report Out-Patient Department 2018, Baguio General Hospital & Medical Center. (Unpublished).
2. Olowookere SA, Ibekwe TS, Adeosun AA. Pattern of tympanic membrane perforation in Ibadan: a retrospective study. *Annals of Ib Postgrad Med.* 2008 Dec;6(2):31-3. DOI:10.4314/aipm.v6i2.64048; PubMed PMID: 25161451: PubMed Central PMCID: PMC 4111000.
3. Adegbiji WA, Olajide GT, Olajuyin OA, Olatoke F, Nwawolo CC. Pattern of tympanic membrane perforation in a tertiary hospital in Nigeria. *Niger J Clin Pract.* 2018 Aug;21(8):1044-1049 DOI: 10.4103/njcp.njcp_380_17; PubMed PMID: 30074009.
4. Yang J, Lou ZH, Lou ZC. A retrospective study of EGF and ofloxacin drops in the healing of human large traumatic eardrum perforation. *Am J Otolaryngol.* 2016 Mar; 37(4):294-298 DOI:10.1016/j.amjoto.2016.03.005; PubMed PMID: 27105974.
5. Dvorak DW, Abbas G, Ali T, Stevenson S, Welling DB. Repair of chronic tympanic membrane perforations with long-term epidermal growth factor. *Laryngoscope.* 1995 Dec; 105(12 Pt 1):1300-04. DOI:10.1288/00005537-199512000-00007; PubMed PMID: 8523981.
6. Lou ZH, Lou ZI, Tang Y. Comparative study on the effects of EGF and bFGF on the healing of human large traumatic perforations of the tympanic membrane. *Laryngoscope.* 2016 Jan; 126(1):E23-8. DOI: 10.1002/lary.25715; PubMed PMID: 26451761.
7. Bhat VK, Veetil BV, Mathad VK. Effect of silver nitrate and epidermal growth factor on nonhealing tympanic membrane perforations: a randomized controlled study. *Ann Otol Neurotol.* 2018;1(1):29-33. DOI: 10.1055/s-0037-1612644.
8. Lou ZC, Lou Z. Efficacy of EGF and Gelatin Sponge for Traumatic Tympanic Membrane Perforations: A Randomized Controlled Study. *Otolaryngol Head Neck Surg.* 2018 Dec;159(6): 1028-1036 DOI: 10.1177/0194599818792019; PubMed PMID: 30060707.
9. Afshari M, Larijani B, Fadayee M, Darvishzadeh F, Ghahary A, Pajouhi M, et al. Efficacy of topical epidermal growth factor in healing diabetic foot ulcers. *Therapy.* 2005; 2(5) 759-65. DOI:10.1586/14750708.2.4.759.
10. Amoils CP, Jackler RK, Lustig LR. Repair of chronic tympanic membrane perforations using epidermal growth factor. *Otolaryngol Head Neck Surg.* 1992 Nov; 107(5) 669-83. DOI: 10.1177/019459989210700509; PubMed PMID: 1437205.
11. Nayak PS, Harugop AS, Patil PH, Prasad TVRK, Goswami L. Treatment of tympanic membrane perforation with topical epidermal growth factor: progress towards clinical application. *Int J Otorhinolaryngol Head Neck Surg.* 2021 May;7(5):768-71. DOI: http://dx.doi.org/10.18203/issn.2454-5929.ijohns20211567.
12. Santos F, Shu E, Lee DJ, Jung D, Quesnel A, Stankovic KM, et al. Topical fibroblast growth factor-2 for treatment of chronic tympanic membrane perforations. *Laryngoscope Investig Otolaryngol.* 2020 May; 5(4): 657-65. DOI: 10.1002/lio2.395; PubMed PMID: 32864435; PubMed Central PMCID: PMC7444771.