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ORIGINAL RESEARCH

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Topical fibroblast growth factor-2 for treatment of chronic tympanic membrane perforations

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Abstract

Objective: To determine the efficacy of fibroblast growth factor-2 (FGF-2) in treating chronic nonhealing tympanic membrane (TM) perforations.

Method: Double-blinded, randomized placebo controlled phase 2 clinical trial for patients with chronic TM perforations of more than 3 months duration with a cross-over arm. Patients received either FGF-2 or placebo (sterile water) saturated gelatin sponge in the perforation after rimming the perforation under topical anesthesia. The perforation was then covered with Tisseel fibrin glue. The primary endpoint was complete closure of the TM perforation. Secondary end points included change in hearing and partial TM closure rates. The TM was examined every 3 weeks with otoendoscopy for closure. The treatment was repeated if there was incomplete closure every 3 weeks up to a total of three treatments per arm.

Results: Seventy four patients were recruited for the study. Fifty seven met eligibility criteria and fifty four completed the study. Ten of 14 perforations closed completely in the placebo group (71.4%) and 23 of 40 perforations closed completely in the FGF-2 treatment group (57.5%), *P* value = .36. Pure tone averages and word recognition scores were not statistically significantly different between study groups post-treatment. After initial complete closure, re-perforation occurred in seven FGF-2 treated patients and two placebo patients making the effective final closure rate 40% for FGF and 57% for placebo, respectively.

Conclusion: No statistically significant difference in tympanic membrane perforation closure rate was found between the FGF-2 and placebo groups. There were no differences in hearing outcomes between the groups.

Level of evidence: 1b.

KEYWORDS

basic FGF, bFGF, chronic tympanic membrane perforation, FGF2, fibroblast growth factor, growth factor, tympanic membrane, tympanic membrane perforation

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1 | INTRODUCTION

The intact tympanic membrane (TM) transmits sound from the external ear to the middle ear ossicles and protects the middle ear from the external environment. Perforations of the TM can occur as a consequence of infection, baro- or penetrating trauma, blast injuries, electrical, thermal, and iatrogenic causes. While most perforations will heal spontaneously, some are refractory to spontaneous healing resulting in a chronic perforation. Blast injuries and burns from slag or lightening are particularly recalcitrant to spontaneous healing.[1,2]

Chronic perforations of the TM can affect hearing and increase the likelihood of acute and chronic otitis media, conductive hearing loss, tympanosclerosis formation, and rarely sensorineural hearing loss, balance dysfunction, meningitis, or brain abscess. Current common treatments of chronic TM perforations include observation with water precautions or surgical repair.

The use of topically applied growth factors to promote tympanic membrane closure has been suggested as an attractive alternative to conventional tympanoplasty. Previous animal studies showed a high rate of chronic TM perforation closures with topically applied growth factors.[3,4] Previous clinical studies have also been encouraging. Hakuba et al in 2010 reported a 92% complete closure rate and near total closure in 5.7% of chronic tympanic membrane perforations with application of topical FGF-2 with an atelocollagen/silicone bilayer membrane.[5] Likewise in 2011, Kanemaru et al reported 98% complete TM perforation closure rate with no complications with the topical application of FGF-2 applied to impregnated gelatin sponges in 53 patients.[6] Only 10% of control patients obtained complete closure.

In the United States, there are currently no FDA-approved drugs for nonsurgical treatment of tympanic membrane perforations. The only study to date looking at chronic perforations of mixed etiology while randomized was not double-blinded.[6] The present study was undertaken to further minimize treatment bias by means of a double-blinded, randomized placebo controlled phase 2 clinical trial in a US cohort of patients with chronic TM perforations of more than 3 months duration.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was a double blinded, placebo controlled phase II study with a cross-over arm. The study was approved by the Massachusetts Eye and Ear Institutional Review Board (2019P000592) and written informed consents were obtained from all patients. The documentation of tympanic membrane (TM) closure was the primary outcome measure. The study was divided into two phases, the Randomized Treatment phase (part A), and the Unblinded Crossover phase (part B). In part A of the study, patients were randomized 3:1 to receive FGF-2 or placebo treatment for up to three treatments separated by 3 weeks intervals. Patients who failed three study treatments moved on to part B of the study. Patients who received placebo in part A and failed three placebo treatments crossed over to receive unblinded FGF-2 for up to three treatments. Patients who received FGF-2 in part A and failed three experimental treatments did not have additional FGF-2 treatment, and moved on to study follow-up.

Research Randomizer was used to randomly assign patients to unequal treatment group in a 3:1 ratio.[7] A random number scheme was created containing 15 sets of numbers with four unique numbers per set (1-4). Of the four numbers per set, patients assigned to numbers 1, 2, or 3 received FGF-2 and patients assigned to number 4 received placebo.

The primary outcome for the study was to determine the TMP closure efficacy of FGF-2 and placebo, with up to three treatments each. The optimal biologic dose was previously determined to be 100 μg of FGF-2 per 1 mL with an estimated dose of about 20 μg in 0.2 mL.[6]

An interim analysis for efficacy was performed on 11 June 2018 and analyzed using a Haybittle-Peto approach with a type I error of 0.001.

Secondary outcomes for the study included: (a) measurement of pure-tone and speech discrimination scores and (b) determination of partial closure of perforation compared to pretreatment.

2.2 | Patients

Seventy four patients suffering from chronic tympanic membrane perforations were recruited from the Massachusetts Eye and Ear Otology clinic. Inclusion criteria covered patients who were over 18 years old and had a dry membrane perforation for greater than 3 months duration. Etiologies of perforations included trauma, transtympanic injections, blast injuries, Eustachian tube dysfunction, infection, and other unknown etiologies. Patients with active otitis media and chronic otorrhea were excluded, as were those with inadequately managed diabetes (HbA1c 6.0% or higher). A total of 57 patients qualified for study and received at least one treatment, and 54 patients completed all treatments. Thirty five male and 39 females were consented, with a mean age of 51.1 years.

2.3 | Study visits

All surgeons participating in this study were neurotologists. All participated live and video training in the technique by Professor Kanemaru. In an outpatient examination, the subject was confirmed to have a tympanic membrane perforation without active infection and or inflammation by microscopic otoscopy. An in office myringoplasty was performed after a pretreatment photograph was taken of the tympanic membrane. After the application of topical 4% lidocaine jelly to the surface of the tympanic membrane for 15 minutes, the edge of the TM perforation was postage stamped with a micro-pick to remove the epithelium circumferentially around the perforation edge. Human fibroblast growth factor-2 (FGF-2) soaked pledgets (approximately 20 μg in 0.2 mL) of bio absorbable gelatin sponge were then placed

through the tympanic membrane perforation into the middle ear until the pledgets completely covered the area under the perforation. For the placebo group, only sterile water was delivered by the pharmacy for saturation of the gelatin sponge. The pledgets and perforation were then covered laterally with Tisseel fibrin glue. Patients were released with instructions to avoid: violent blowing of the nose, flying, swimming, and getting water in their ears.

The subject returned to the clinic at 3 weeks (±7 days) for a follow-up appointment. An audiogram was performed to assess changes in pure-tone and speech discrimination scores. The tympanic membrane was examined to determine the status of the perforation and photographed in the same fashion as during the pre-treatment visit. If the tympanic membrane closed, the subject was seen again at 2 months (±7 days) for a final study visit. If the perforation did not close, the FGF-2 or placebo application was duplicated in the same fashion as the initial administration visit and the tympanic membrane assessed again at 3 weeks (±7 days).

If the perforation remained open after three unblinded treatments, the blind on the subject's randomization status was broken. If the patients were randomized to placebo, they were crossed over to receive unblinded FGF-2 treatment. If the patients were randomized to FGF-2 treatment, they received no further study treatment and returned in 2 months (±7 days) for the final follow-up visit. At the final visit, the TM was examined for closure. Patients also received a final audiogram with tympanometry to assess the mobility of their TM.

3 | RESULTS

Seventy four patients signed informed consent to participate in this study. Fifty seven patients (77.0%) met all the eligibility criteria and had at least one treatment. Fifty four patients (94.7% of those eligible) completed all treatment visits while three patients withdrew from the study. Twenty nine patients were female (53.7%) and 25 patients were male (46.3%). Patients were compared between treatment groups: FGF2 vs placebo (Figure 1). Only the data from the 54 patients who completed all treatment visits were used for analysis (Figure 2). Etiologies of perforations included trauma, transtympanic injections, blast injuries, eustachian tube dysfunction, infection, and other unknown etiologies.

There were 42 patients (73.7%) randomized to FGF2 and 15 patients (26.3%) to placebo. A subgroup of four patients was crossed-over from placebo to FGF2 after three unsuccessful treatments with placebo. Two patients in the FGF2 group and one patient in the placebo group withdrew from the study prior to completion.

The interim analysis failed to provide evidence of efficacy (*P* value = .15) so the study was continued.

3.1 | Adverse events

In the FGF group, five patients (11.9%) experienced ear drainage post-treatment; three patients (7.1%) complained about ear pain during the procedure; and five patients (11.9%) experienced infections. Out of the five patients who had ear infections, two of them had more than one infection treated with topical antibiotics, but only one patient was withdrawn from the study for infection.

In the control group, two patients (13.3%) experienced ear drainage post-treatment; two (13.3%) patients complained about ear pain during the procedure; and one patient (6.7%) experienced recurrent fungal infections.

There were no adverse events during the cross-over phase for the four patients that were unblinded and crossed-over from placebo to FGF.

In terms of adverse event management, one patient from the control group and one patient from the FGF group were withdrawn due to infection as noted above. The patient from the FGF group had one instance of infection deemed unrelated and the second occurrence as possibly related; the infection in the other patient from the control group was deemed unrelated to the study drug as the patient had a history of fungal ear infections. Both patients followed up with their otology physicians after being taken off-study. One patient from the FGF group withdrew due to travel requirements and was lost to follow-up.

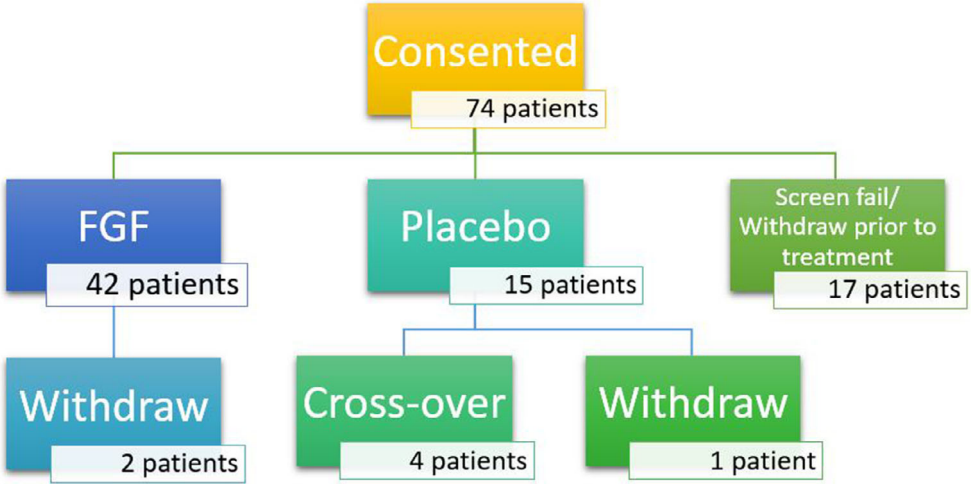

FIGURE 1 Enrollment, randomization, and screen fail/withdrawal of study participants

FIGURE 2 Tympanic membrane closure by randomization group. Change in perforation size determined by comparing baseline perforation to perforation at the end of the study

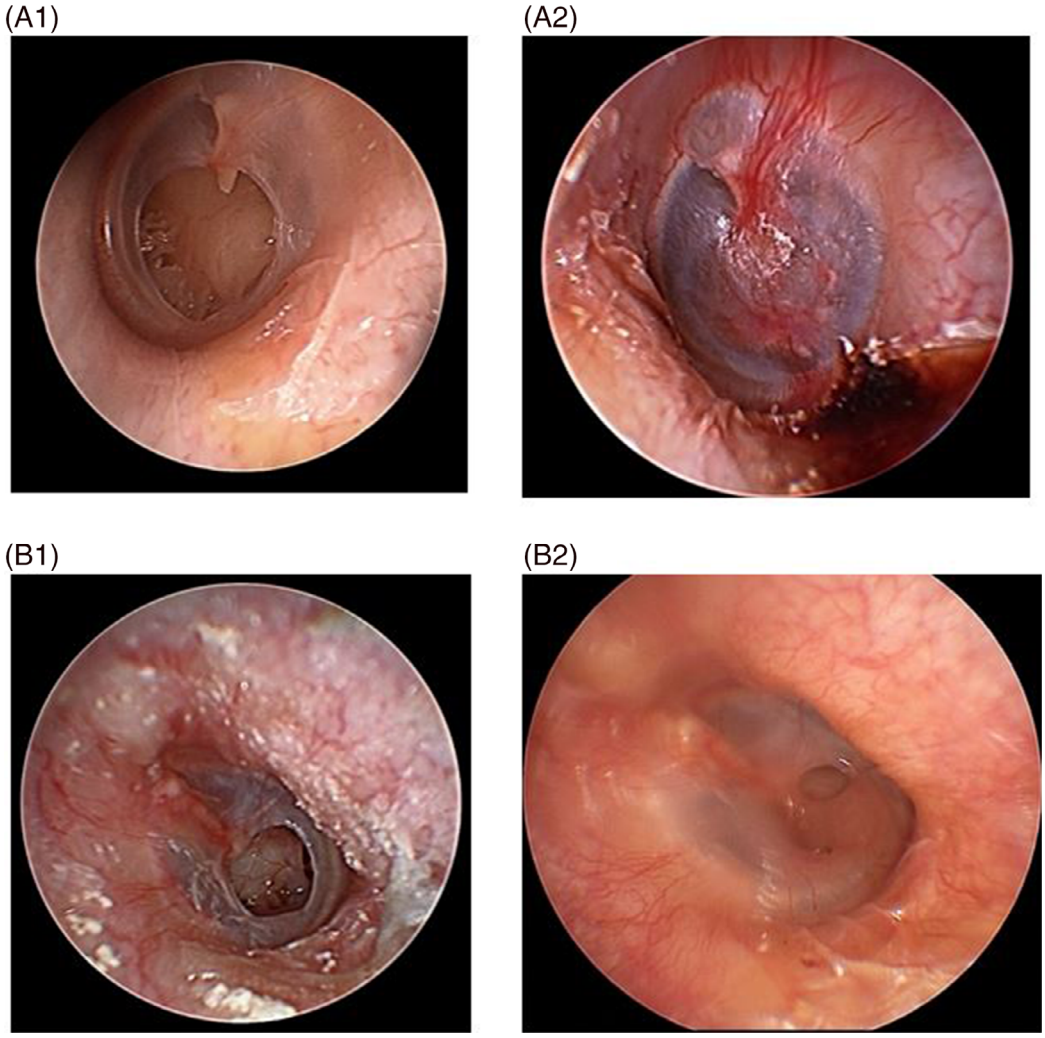

FIGURE 3 TM treated with FGF2. A, FGF2-treated patient with closure. A1, Pre-FGF2 treatment. A2, Post-1x FGF2 treatment and TM closure. B, FGF2 treated patient without closure. B1, Pre-FGF2 treatment. B2, Post-3x FGF2 treatment with remaining TM perforation

3.2 | Tympanic membrane perforation closure

Twenty three of 40 perforations closed in the treatment group for a closure rate of 57.5%. Fourteen of 17 perforations that did not heal got smaller when compared to baseline perforation size (82.4%). In total, 37 out of 40 perforations responded favorably to FGF treatment (92.5%; Figure 2). Fourteen patients' perforations closed after one treatment visit, eight closed after two treatment visits, and one closed after three treatment visits. Endoscopic images of closure and non-closure TM were taken (Figure 3).

Ten of 14 perforations closed in the placebo group for a closure rate of 71.4%. Three of four perforations that did not close got smaller when compared to baseline perforation size (75%). Thirteen out of 14 perforations responded favorably to placebo (92.8%) (Figure 2). Four patients' perforations closed after one treatment visit, five patients' perforation closed after two treatment visits, and one

patient's perforation closed after three treatment visits. Endoscopic images of closure and nonclosure TM were taken (Figure 4).

A total of 54 patients who completed the study were included in the analysis (Table 1). The two treatment groups' closure rates were not significantly different (*P* value = .36).

Two of four patients who were crossed-over from placebo to FGF group closed after three placebo treatments and three FGF treatments (50%). The other two patients' perforations did not close or get smaller when compared to baseline perforation size. When we analyze the cross-over group, the total number of patients that received FGF treatment becomes 44, and closure rate is then 56.8%.

3.3 | Hearing outcomes

Hearing outcomes were evaluated based on the changes in pure-tone averages (PTA) and word recognition scores (CNC) from baseline to the completion of the study. PTA was calculated using 0.5, 1, 2, and 3 kHz air conduction thresholds. When the threshold at 3 kHz is missing, then it is interpolated by averaging the thresholds at 2 and 4 kHz.

In the FGF treatment group, the 23 patients with complete closure of their perforations had a mean improvement of 8.4 dB in PTA and 1% increase in CNC; 14 patients with subtotal closures had a mean improvement of 6.9 dB in PTA and 4.4% increase in CNC; and 3 patients with no change in their perforations had an average improvement of 9.7 dB in PTA but a 6% decrease in CNC.

In the placebo treatment group, 10 patients with complete closure of their perforations had a mean improvement of 5.7 dB in PTA and 1% increase in CNC; 3 patients with subtotal closures had a mean improvement of 8.7 dB in PTA and 5.3% increase in CNC, and 1 patient had an improvement of 4 dB in PTA and no change in CNC.

For patients who were crossed-over from placebo to FGF, two patients whose perforations closed completely had a PTA improvement of 18.5 dB and an increase of 7% in CNC, while the other two

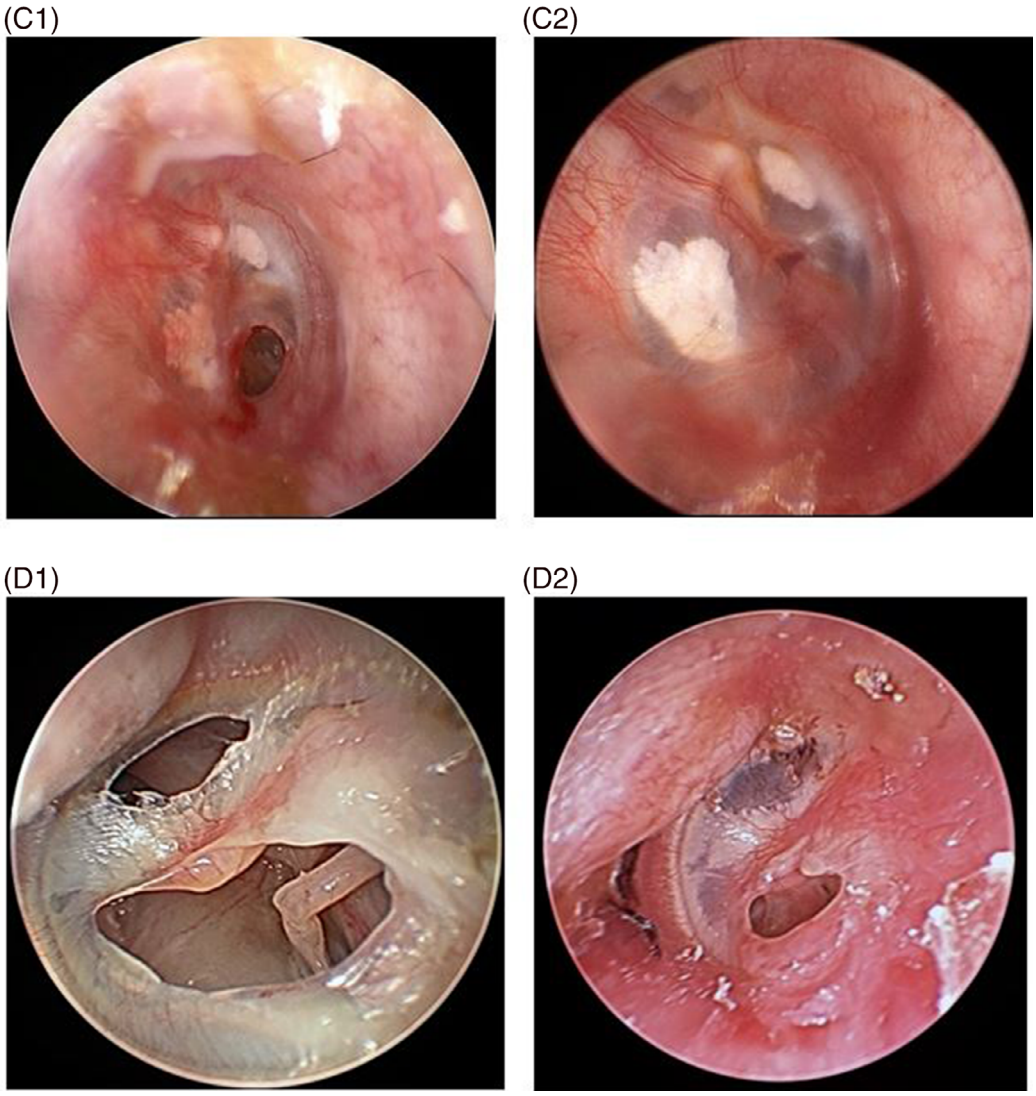

FIGURE 4 TM treated with Placebo. C, Placebo-treated patient with closure. C1, Pre-Placebo treatment. C2, Post-2x Placebo treatment and TM closure. D, Placebo-treated patient without closure. D1, Pre-Placebo treatment. D2, Post-3x Placebo treatment with remaining TM perforation. This TM had two perforations, both were treated with placebo and one closed, and one got smaller

TABLE 1 Demographics of 54 patients who completed the study and were included in the analysis

	FGF (n = 40)	Placebo (n = 14)	Total (n = 54)
Gender			
Male	18	7	25
Female	22	7	29
Age (years)			
Mean (SD)	51.2 (14.2)	56.3 (16)	52.5 (14.7)
Median	51	60	52.5
Min, Max	20, 76	32, 82	20, 82
Etiology			
Infection	13	5	18
ETD	1	1	2
TM tube/injection	12	2	14
Blast/trauma	6	0	6
Diving/swim	4	1	5
Stifled sneeze	1	1	2
Born with	1	0	1
Unknown	2	4	6
Perforation			
Average start size	12%	11%	12%
# of closure	23	10	33
# of nonclosure	17	4	21
Average % reduction in nonclosure patients	−8%	−8%	−8%
Duration of perforation			
Estimated (months), mean (SD)	48.1 (134.3)	47.6 (95.5)	48 (125.4)

patients who had no change in their perforations had a decrease in PTA of 3.5 dB and 1% increase in CNC.

After adjusting for baseline PTA, FGF had a 1.34 dB lower mean PTA score than placebo at follow-up with a *P* value of .64 (95% CI −7.12 to 4.44). Removing three influential patients in a sensitivity analysis had minor effects on the results. FGF had no impact (0.0, *P* value = 1.0) on the median CNC score at follow-up compared to placebo after adjusting for baseline CNC. Therefore, no evidence of an association between study group and follow-up hearing results was found.

3.4 | Exploratory endpoints

We considered the characteristics of the perforation and multiple surgeons performing the study procedures as potential confounders. The variables that we explored were the history of prior tympanoplasty, starting size of the perforation, etiology of the perforation (infectious vs noninfectious), and surgeon dependence.

Of the 54 patients who completed the study, 17 individuals (31.5%) had prior tympanoplasty and 37 (68.5%) did not. Expressed as an odds ratio, the primary outcome measuring the effect of study group on perforation closure was 0.54 (95% CI 0.15-2.02, *P* value = .36), suggesting that the odds of perforation closure are lower in the FGF group than the placebo group. The resulting *P* value for an interaction between prior tympanoplasty status and study group was .10, with the odds ratio for study group among those with prior tympanoplasty being 0.07 (95% CI 0.002-1.94) and the odds ratio for study group among those without prior tympanoplasty being 1.44 (95% CI 0.31-6.61). Therefore, we failed to find evidence of a difference in the effect of study group by tympanoplasty status.

No evidence was found that baseline perforation size had an effect on closure by study group (*P* value for interaction = .73). The number of patients treated per surgeon was too variable to determine the effect of performing surgeon on closure outcome (Appendix S1).

Data were pooled across study groups to assess the association between etiology (n = 20 infectious etiology, n = 28 noninfectious etiology, n = 6 unknown) and TM closure. The three individuals who withdrew and six individuals with unknown infection status were dropped from this analysis (n = 48 used). The odds ratio for the association between perforation closure and etiology was 0.58 (95% CI 0.18-1.89) with a *P* value of .37. The odds ratio suggests that having an infection decreases the odds of closure, but the *P* value is not statistically significant.

3.5 | Re-perforations

Seven FGF-2 patients and two placebo patients whose TM perforations closed completely after treatment were later found to have a re-perforation of the TM. The majority occurred within 2 months. Our stated endpoint at the onset of the study was complete tympanic membrane closure determined by otoscopic exam and tympanometry at any point following treatment, resulting in 57.5% for FGF-2 and 71.4% for placebo. However, the effective final closure rate was poorer at 40% for FGF-2 and 57% for placebo, respectively.

4 | STATISTICAL ANALYSIS

The primary outcome of the study (perforation closure during follow-up) was tested using a Pearson's chi-squared test. The effect of study group on PTA score was analyzed using ordinary linear regression. Baseline PTA was adjusted for linearly in the model in order to improve power. Normality of residuals was appropriate, but heteroscedasticity-consistent SEs were used to account for nonconstant variance. As a sensitivity analysis, three influential participants with high Cook's distance values were removed. The effect of study group on CNC score was analyzed using quantile regression. This approach was used due to CNC being measured as a proportion and being highly left skewed and near 1.0. As with PTA, the baseline response was adjusted for. As an exploratory analysis, binary logistic regression was used to determine if the effect of study group on perforation closure varied by prior tympanoplasty status through the use

of a multiplicative interaction term. Due to the occurrence of quasi-complete separation, Firth's correction was utilized. Logistic regression was also used to determine if the effect of study group on closure varied by baseline perforation size through the inclusion of a multiplicative interaction term. Lastly, logistic regression was used to determine if etiology (infectious or noninfectious) was associated with closure. *P* values less than .05 were considered statistically significant and all tests were two-tailed.

5 | DISCUSSION

Human FGF-2 or basic FGF has been the most studied FGF isoform and its production and clinical applications in wound healing are well described.[8] Basic fibroblast growth factor (B-FGF) is a mitogen. It is known to promote mitoses for endothelial cells, smooth muscle cells, chondrocytes, keratinocytes, fibroblasts, and other mesenchymal cells. It has therefore been used in studies to determine its effects on wound healing. FGF receptors (FGFR) 1 to 4 have been localized within the epidermal and mucosal layer of human tympanic membranes from the rim of perforations.[9] Progenitor/stem cells were detected in the basal layer of keratinizing epithelium around the umbo and in the peri-annular regions in humans.[10]

FGF was first shown to favorably promote closure of tympanic membrane perforations in animal models. The use of FGF-2 in rat and guinea pig shows a rapid healing response characterized by hypertrophic subepithelial connective tissue reactions.[11,12] The reaction is histologically characterized by neovascularization, deposition of intracellular matrix, collagen fibers, and fibroblasts.

As noted above, several human studies using FGF-2 to promote tympanic membrane perforation closure showed highly promising results. We must ask why the gross disparity in this study. The Kanemaru study applied up to four courses of treatment and we used a maximum of three applications of FGF-2.[6] In the present study, 92.5% of FGF-2 treated patients responded favorably with the perforations becoming smaller, and an additional treatment may have added to our total closure numbers, however, our placebo group also responded in a highly favorable fashion with 92.8% complete or partial closure rate, thus calling into question the role of active FGF-2 in this process.

Prior studies of FGF-2 have not admitted patients who had undergone prior surgical tympanoplasty on the basis that progenitor cells at the annulus or umbo may have been damaged.[6,7,12] While this seems theoretically a potential cause for decreased success, we did not show a difference between patients who had a prior surgical attempt at repair from those patients who had not undergone prior tympanoplasty.

Interestingly, in a follow-up retrospective study by Hakuba et al reported 116 patients with perforations of at least 1-year duration prior to FGF-2 and atelocollagen treatment found a complete closure rate of 62%, which is much closer in outcome to our current study.[12] We found no significant difference in the use of FGF-2 to placebo.

While we cannot exclude a biological effect of FGF-2, the current study does not support its use as described given its nonsuperiority to placebo. Placebo closure rates of 71.4% in the present study suggests that in-office gelatin sponge myringoplasty with fibrin glue may be a suitable treatment option for the management of chronic perforations. Whether fibrin glue has a biological effect that promotes wound healing in addition to mechanical disruption of the perforation margins merits further investigation.

Both placebo and FGF treatment options were associated with few adverse events. Both placebo and treatment groups showed improvement in hearing outcomes. Hearing improvement was observed in both total and subtotal closures. There were no instances of hearing loss. Infection occurred in five patients resulting in withdrawal of two patients.

Re-perforation once healing has occurred has not been previously reported. It is plausible that the closure of the perforations seen which reopened were caused by stimulation only of the epithelial and mucosal layers without fibrous ingrowth in the central fibrous layers as desired. Perhaps the FGF2 receptors seen primarily in the epithelial and mucosal layers of human perforations are a clue into the temporary healing seen in our patients who subsequently re-perforated.[9]

6 | CONCLUSION

We found no statistically significant difference in tympanic membrane perforation closure rate between placebo and FGF. Etiology of perforation, prior tympanoplasty, and size of the perforation could not account for the observed findings. Additional study to fully understand the timing of the expression of growth factors and the influence of progenitor cells in repair of tympanic membranes may result in improved topical treatments for chronic tympanic membrane perforations.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial or nonfinancial interests in the subject matter or materials discussed in this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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