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The Effectiveness of bFGF in the Treatment of Tympanic Membrane Perforations: A Systematic Review and Meta-Analysis

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Objective: To investigate the effectiveness of basic fibroblast growth factor (bFGF) versus placebo or no intervention in the treatment of tympanic membrane (TM) perforations from randomized controlled trials (RCTs), prospective and retrospective studies.

Data Sources: PubMed, EMBASE, and Cochrane databases were screened from their inceptions to June 2019.

Study Selection: Inclusion criteria: 1) English language; 2) observational (retrospective or prospective) or treatment (RCT) studies; 3) reported the outcomes on the application of bFGF in adult or pediatric population. Exclusion criteria: 1) studies without a control group; 2) animal studies, in vitro studies, review studies, and case reports.

Data Extraction: Number of patients, cause of TM perforation, perforation size, treatment, mean age, follow-up time, sex, closure rate, healing time, mean air-bone gap improvement.

Data Synthesis: A total of 14 studies were included, including seven RCTs and seven non-RCTs with a total of 1,072 participants. The odds ratio for closure rate of bFGF treatment was 7.33 (95% confidence interval [CI], 4.65 to 11.53; $p < 0.01$; $I^2 = 44\%$) and the standardized mean difference (SMD) for healing time was −5.89 (95% CI: −7.85 to −3.93, $p < 0.01$, $I^2 = 98\%$), suggesting bFGF application has a significant effect on closure of TM perforations. However, no significant change in hearing (SMD: 0.08, 95% CI: −0.11 to 0.27, $p = 0.39$, $I^2 = 0\%$) was seen as a result of bFGF treatment.

Conclusions: Our meta-analysis has revealed that the application of bFGF can significantly enhance the closure rate as well as shorten the healing time for TM perforations. In terms of hearing, there is as yet no evidence that bFGF has a significant effect. Given its ease, availability, and safety, bFGF can be used effectively for TM repair.

Key Words: Basic fibroblast growth factor—Healing—Hearing—Meta-analysis—Tympanic membrane perforation.

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Tympanic membrane (TM) perforation is commonly encountered in the clinical setting. It can be iatrogenic, or caused by trauma or infections. In the United States, it is estimated that 1 to 3% of the population will have a TM perforation some time in their life (1). In specific groups (e.g., Indigenous Australians), the prevalence reported

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This study was conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration.

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has been as high as 28 to 43% (2). Some perforations may lead to chronic otorrhea and hearing loss, and limit water sports, which may significantly reduce the quality of life (3). Moreover, chronic TM perforations, if left untreated, serve as a conduit for further infections, acquired cholesteatoma, facial nerve palsy, labyrinthitis, and subperiosteal abscess (3,4). Although traumatic TM perforations tend to heal spontaneously, large or chronic perforations may fail to heal and require surgical interventions (5). In addition, the long-term spontaneous healing rate can sometimes be unsatisfactory (6,7). Kristensen (8) reported that healing rate for spontaneous perforations to be 78.7%, which appears to vary depending on the cause of perforation. To repair TM perforations, patients may suffer from complications from conventional surgery (9). Hence, it is crucial to explore options that may improve the efficacy of repair (10).

With the advancement in the field of tissue engineering, various bioscaffolds and bioactive molecules have been applied with encouraging results (10,11). Among them, basic fibroblast growth factor (bFGF) is one of the most commonly used bioactive molecules (12). Until recently, a majority of the studies were in animal models. However, in the recent years, we have witnessed a surge in the use of bFGF in human trials (6,7,13–24). Although a majority of trials reported promising benefits, the perforation closure rates varied across the different studies, and there does not appear to be a consensus for hearing benefits. Recently, a literature review (12) supported the role of bFGF in the repair of TM perforation, but to the best of our knowledge, no quantitative analysis has been performed to evaluate the effectiveness of bFGF.

Thus, the aim of this comprehensive meta-analysis was to assess the effectiveness of bFGF for the repair of TM perforations both in healing and hearing by evaluating the current best evidence.

METHODS

We followed the population, intervention, comparison, outcomes (PICO) format (25). Our study question was: for people with TM perforations, can the use of bFGF improve both the healing rate and time, and hearing outcomes? Our review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (26) (Fig. 1).

Search Strategy and Study Selection

Two authors (J.H. and B.M.T.) independently searched PubMed, EMBASE, and Cochrane databases from establishment to June 2019. Abstracts were identified by the following search terms: 1) tympanic membrane(s); membrane(s), tympanic; eardrum(s) or 2) tympanic membrane perforation; membrane perforation, tympanic; eardrum perforation; perforation, eardrum; tympanic membrane rupture; membrane rupture, tympanic; rupture, tympanic membrane, and 3) fibroblast growth factor 2; basic fibroblast growth factor; class II heparin-binding growth factor; heparin-binding growth factor class II; HBGF-2; prostatropin; FGF-2; fibroblast growth factor, basic; fibroblast growth factor-2; cartilage-derived growth factor; cartilage derived growth factor; prostate epithelial cell growth factor. The original articles were all from peer-reviewed scientific journals published in English. The database search yielded 169 studies, of which 91 studies met the requirements after duplicates were removed.

Inclusion and Exclusion Criteria

After searching, the 91 articles were independently evaluated by two authors (J.H. and B.M.T.) with the following inclusion criteria: 1) observational (retrospective or prospective) or treatment (randomized controlled trials [RCTs]) studies; 2) studies that reported the outcomes on the application of bFGF in adult or pediatric populations. Studies without a control group were excluded. Animal studies, in vitro studies, review studies, and case reports were excluded as well. A third author (Y.S.) was available when any disagreement presented between these two authors. A total of 14 studies were selected for further analysis from the 91 studies through screening the abstract and full texts. Subsequently, the quality of the included studies was judged according to the Cochrane Collaboration's tool for assessing the risk of bias (25) through Review Manager 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark) (Fig. 2).

Outcome Measures

The primary outcome measure was the complete closure rate in the bFGF treatment group versus the complete closure rate in the control group. The secondary outcomes were the differences in healing time and improvement in hearing. The following data was taken or derived from the full report of the 14 studies for both the treatment and control groups: 1) number of subjects, 2) percentage closure, 3) mean and standard deviation (SD) of closure time in days, 4) mean and SD of four frequency (0.5, 1, 2, and 4 kHz) average of air-conduction threshold in decibels (dB) obtained via standard pure-tone audiometric testing. Also recorded were 1) first author, 2) year of publication, 3) the study design (RCT or non-RCT), and 4) size of perforation targeted by the study.

Statistical Analysis

Statistical analyses were performed using STATA version 12 (StataCorp LP, Texas City, TX) and Review Manager version 5.3. Closure rates are expressed as odds radio (OR), while healing times and hearing improvement are reported as the standardized mean difference (SMD). Moreover, the I^2 statistic was applied to assess heterogeneity, with an I^2 value of 25, 50, and 75% indicating a low, moderate, and high heterogeneity respectively (26). All data were dropped into the pool with suitable statistical models. The subgroup analysis was also continued considering that the perforation size has been reported to be associated with healing time (27,28). We divided the studies into three subgroups based on the patients' perforation size, as: large perforation (>50% of the TM); small perforation (<50% of the TM); and unknown size (size of the TM perforation unspecified in the studies) for the outcomes of the healing time. Meanwhile, sensitivity analysis was conducted to ensure reliability of the results by excluding one outlying study. Funnel plots were used to examine publication bias. In addition, Begg's tests were used to quantitatively assess publication bias (29). To further examine the possible effect of publication bias, the trim-and-fill method also was used in our analyses (30). All p values were two sided, with $p < 0.05$ being considered as statistically significant.

RESULTS

Characteristics of the Studies

Of the 91 studies identified, a total of 14 studies with 1,072 patients were included in this meta-analysis, consisting of seven RCTs (7,14,15,17,19,23,24) and seven non-RCTs (6,13,16,18,20–22). The included studies

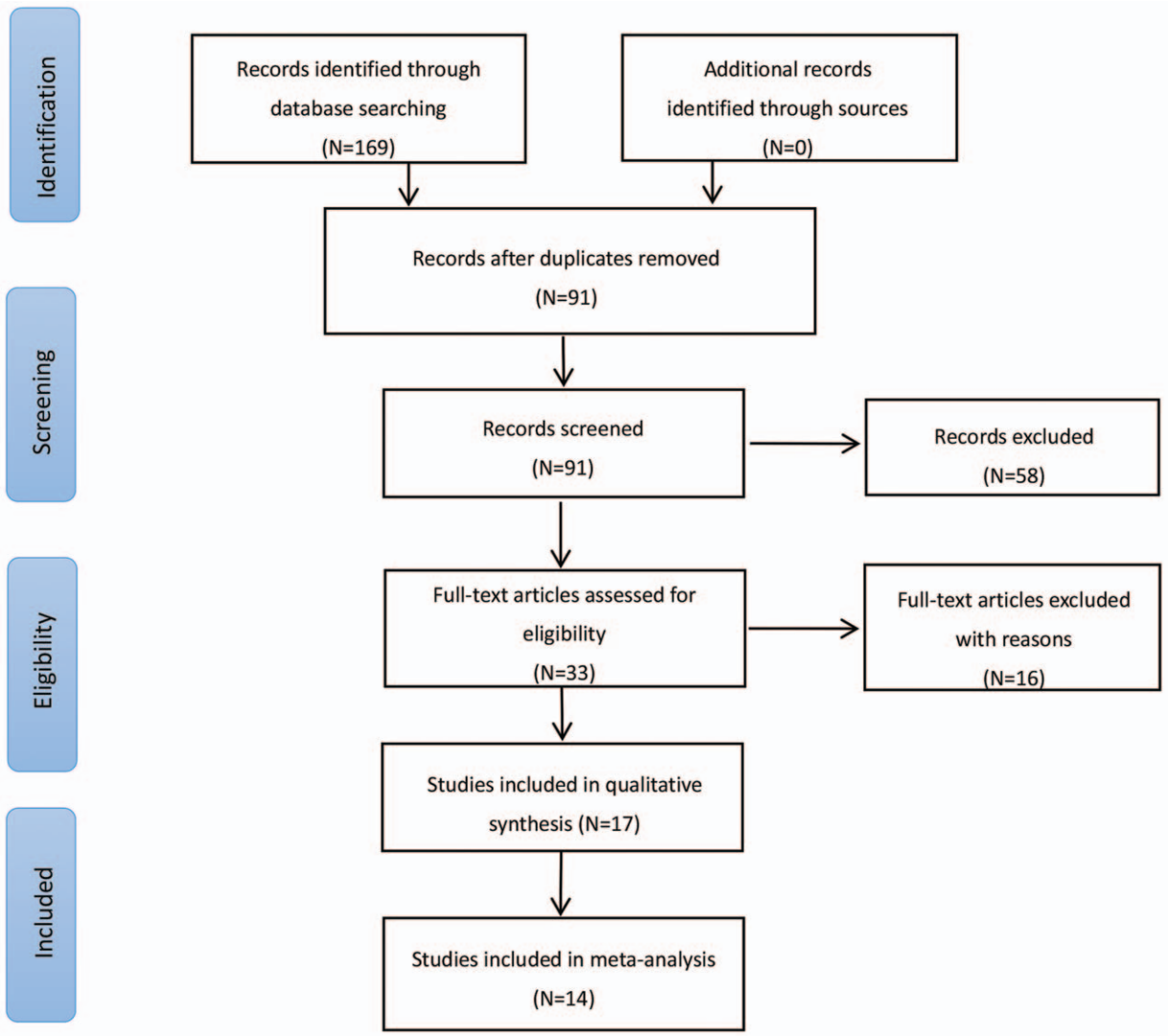

FIG. 1. Flow diagram of the search process and search outcomes.

were published between 2003 and 2018, with a range of follow-up time from 3 to 12 months. Perforations in three studies (6,13,23) were chronic due to various causes, while perforations in the other studies (7,14–22,24) were caused by trauma. All studies reported the closure rates (6,7,13–24); only eight studies reported the healing time (7,15,18–22,24). Six studies provided the outcomes of hearing (7,14,15,19,21,24). The characteristics and statistics of the selected documents were summarized in Table 1. The detailed information of the bFGF application in each study was also summarized in Supplemental Table 1, http://links.lww.com/MAO/A966, including composition, manufacturer, concentration, doses, frequency, and duration.

Effectiveness of bFGF on Closure Rate in Acute and Chronic TM Perforations

All articles that fulfilled the selection criteria were included in the statistical tool to calculate the OR value with a fixed-effects model. A forest plot demonstrates the differences (Fig. 3). For acute perforations consisting of 11 studies, the closure rates ranged from 89 to 100% (median 97.5%) in the bFGF groups, while it ranged from 53 to 90% (median 79.5%) in the control groups (Table 1). For chronic perforations, two studies demonstrated closure rates of 98 and 100% in the bFGF groups, compared with 10 and 40% in their control groups, respectively (6,23). Another study by Kanemaru et al. (13) reported a complete closure rate of 91% following bFGF treatment compared with 95% in the control group undergoing standard type I tympanoplasty with temporal fascia for patients with chronic TM perforations. Analysis of overall closure rate of bFGF treatment revealed an OR of 7.33 (95% CI, 4.65 to 11.53; $p < 0.01$), which indicates a statistically significant improvement when compared with the control group with a moderate heterogeneity ($I^2 = 44\%$). Specifically, the ORs of the subgroup results were 7.20 (95% CI, 4.34 to 11.95; $p < 0.01$,

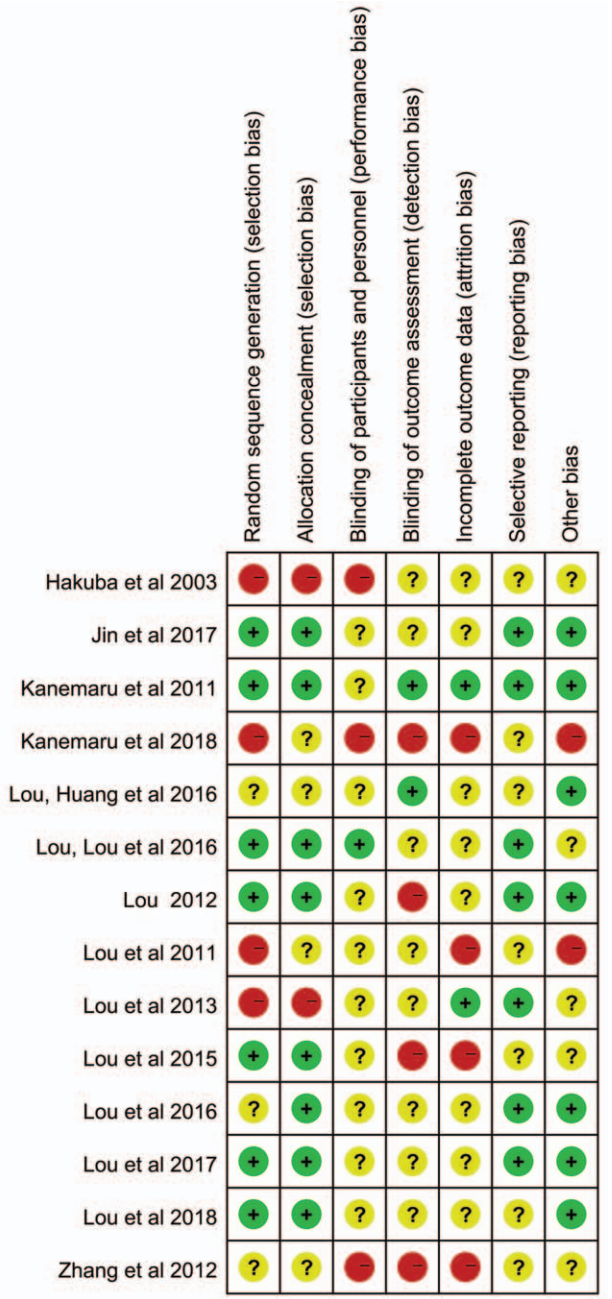

FIG. 2. Risk of bias summary for enrolled studies. Green, yellow, and red circles indicate a low, unclear, and high risk of bias, respectively.

$I^2 = 0\%$) for acute perforations and 8.03 (95% CI, 3.04 to 21.18; $p < 0.01$, $I^2 = 85\%$) for chronic perforations. A sensitivity analysis did not change the results of this meta-analysis. Thus, the results of the data analysis can be considered steady and reliable.

The Begg's funnel plot was applied to estimate potential publication bias (Supplemental Figure 1A, http://links.lww.com/MAO/A967). The result of Begg's test ($p = 0.02$) revealed the possibility of publication bias in reporting closure rate. The trim-and-fill method was used to assess and adjust the publication bias, which hypothesized that publication bias accounts for funnel plot asymmetry. Some negative unpublished studies were used to yield a symmetrical funnel plot (Supplemental Figure 1B, http://links.lww.com/MAO/A967); the transformation still demonstrated no significant change to the pooled ORs (4.22, 95% CI: 2.63 to 6.76).

Effect of bFGF on Healing Time in Acute TM Perforations

Eight studies reported the healing time, however, one of them (15) was excluded due to different reporting

TABLE 1. *Characteristics of studies included in the meta-analysis*

Study	Year	Study Design	Type of TMP	TMP Size (%)	No. of Patients	Age[a] (yr)		Gender (male:female)		Follow-up (mo)	Treatment		Closure Rate (%)		Healing Time[b] (d)		Hearing Improvement[b] (dB)	
						bFGF Group	Control Group	bFGF Group	Control Group		bFGF Group	Control Group	bFGF Group	Control Group	bFGF Group	Control Group	bFGF Group	Control Group
Lou et al. (14)	2018	RCT	Acute	>25	131	37.4	37.4	25:42	29:35	6–12	bFGF	NI	96	73	NA	NA	13.6 ± 2.6	12.7 ± 4.3
Kanemaru et al. (13)	2018	Retrospective	Chronic	NA	65	54	50.1	NA		6	bFGF/V/ Intervention[c]	Surgery[d]	91	95	NA	NA	NA	NA
Jin et al. (24)	2017	RCT	Acute	>25	97	34.5	35.0	16:32	14:35	6	bFGF/V	NI	98	90	15.7 ± 5.1	24.8 ± 4.9	13.3 ± 3.1	14.1 ± 2.7
Lou et al. (15)	2017	RCT	Acute	>25	89	37.68	37.24	16:28	23:22	6	bFGF	NI	93	82	NA	NA	10.89 ± 5.16	9.29 ± 5.36
Lou, Lou et al. (7)	2016	RCT	Acute	>25	57	38.0	37.8	17:11	15:14	3	bFGF	NI	89	72	13.7 ± 7.6	28.1 ± 12.2	12.6 ± 6.4	12.9 ± 5.1
Lou et al. (18)	2016	Prospective	Acute	>25	118	37.7	37.5	33:40	24:21	6	bFGF	NI	93	82	12.3 ± 8.15	25.6 ± 13.32	NA	NA
Lou, Huang et al. (16)	2016	Prospective	Acute	>12.5	29	39.1	35.8	8:4	6:11	6	bFGF	NI	92	53	NA	NA	NA	NA
Lou et al. (17)	2015	RCT	Acute	>25	86	32.4	31.9	15:31	12:28	6	bFGF	NI	98	83	NA	NA	NA	NA
Lou et al. (20)	2013	Prospective	Acute	>50	36	36.8	36.2	9:11	5:11	6	bFGF	NI	100	56	12.4 ± 3.6	48.2 ± 5.3	NA	NA
Lou (19)	2012	RCT	Acute	>50	61	32.8	33.6	12:20	10:19	NA	bFGF	NI	100	55	11.06 ± 1.52	46.25 ± 8.71	12.7 ± 2.9	12.4 ± 3.1
Zhang et al. (21)	2012	Prospective	Acute	<25	93	27.9	25.3	NA		3	bFGF	NI	100	77	12.6 ± 1.2	43.1 ± 2.5	11.7 ± 2.4	11.5 ± 1.9
Kanemaru et al. (23)	2011	RCT	Chronic	NA	63	55		26:30		3	bFGF/V	Saline/V	98	10	NA	NA	NA	NA
Lou et al. (22)	2011	Retrospective	Acute	<50	71	15.3		61:75		NA	bFGF/V	NI	97	86	10.4 ± 2.5	27.3 ± 2.4	NA	NA
				>50	62								100	84	11.1 ± 1.9	28.6 ± 3.1	NA	NA
Hakuba et al. (6)	2003	Prospective	Chronic	NA	14	62.3	54.2	NA		NA	bFGF/V	Saline/V	100	40	NA	NA	NA	NA

[a]Values are presented as mean.
[b]Values are presented as mean ± SD.
[c]Intervention indicates perforation edge freshening.
[d]Surgery indicates standard type I tympanoplasty.
bFGF indicates basic fibroblast growth factor; dB, decibel; NA, data not available; NI, no intervention; RCT, randomized controlled trial; TMP, tympanic membrane perforation; V, vehicles.

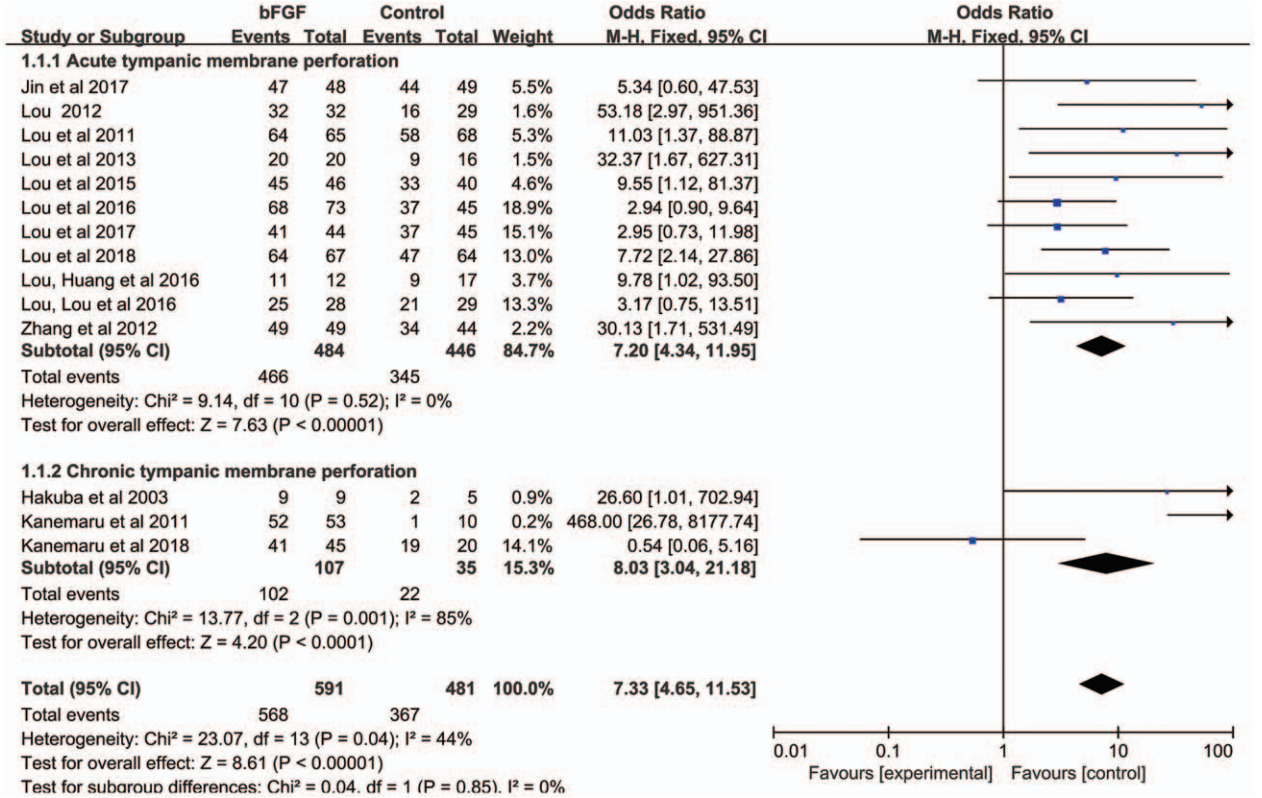

FIG. 3. Forest plot of closure rate between bFGF and control group. bFGF indicates basic fibroblast growth factor; CI, confidence interval; M-H, Mantel-Haenszel.

methods for results compared with the others. The seven studies, all with acute TM perforations, had a total number of 524 participants. The average closure time ranged from 10.4 to 15.7 days in the bFGF groups and 24.8 to 48.2 days in the control groups (7,18–22,24). Overall, healing times were significantly shorter for the bFGF group compared with control group (SMD = −5.89; 95% CI: −7.85 to −3.93, $p < 0.01$) but showed high heterogeneity ($I^2 = 98\%$).

The forest plot showed a random effects meta-analysis for the difference in healing time after the bFGF treatment among these studies (Fig. 4). The healing times in the large perforations (SMD = −7.01; 95% CI: −7.96 to −6.06, $p < 0.01$, $I^2 = 0\%$), small perforations (SMD = −11.53; 95% CI: −20.92 to −2.14, $p = 0.02$, $I^2 = 98\%$), and perforation of unknown size (SMD = −1.50; 95% CI: −1.84 to −1.77, $p < 0.01$, $I^2 = 18\%$) were in each case statistically shorter in the bFGF group compared with the control group. Large perforations and perforations of unknown size were not sources of heterogeneity; instead small perforations may account for the heterogeneity ($p < 0.01$). Sensitivity analysis reflected the reliability of these outcomes.

Effect of bFGF on Hearing Improvement in Acute TM Perforations

Six studies (n = 457 subjects) reporting the hearing improvement were included in a fixed-effect model. All of them consisted of acute TM perforations. To evaluate hearing improvement, the thresholds of pure-tone average were compared before and at the final follow-up following bFGF treatment. There was no statistically significant improvement in hearing (SMD = 0.08; 95% CI: −0.11 to 0.27, $p = 0.39$) in the bFGF treatment group compared with the control group. No significant inter-study heterogeneity was found ($I^2 = 0\%$) (Fig. 5). The conclusion did not change following sensitivity analysis.

DISCUSSION

TM perforations lead to significant morbidities and lifestyle limitations. Although acute TM perforations tend to heal spontaneously, the long-term healing results are suboptimal (8). For chronic TM perforations, surgeries using fascia or cartilage autografts are common practice (5). However, all of these autografts have their own limitations; an ideal graft material is yet to be identified (10,31). Moreover, patients who undergo surgeries undertake the risks of general anesthesia, high medical costs, surgical complications, and loss of productivity (9,12). Hence, various alternatives have been explored to promote TM regeneration. Previous researches indicate that bFGF is a safe, convenient and effective treatment for TM repair (16,19,23). However, the majority studies are outcome-based and subjective in nature. In this study, we have used quantitative meta-analysis to evaluate the effectiveness of

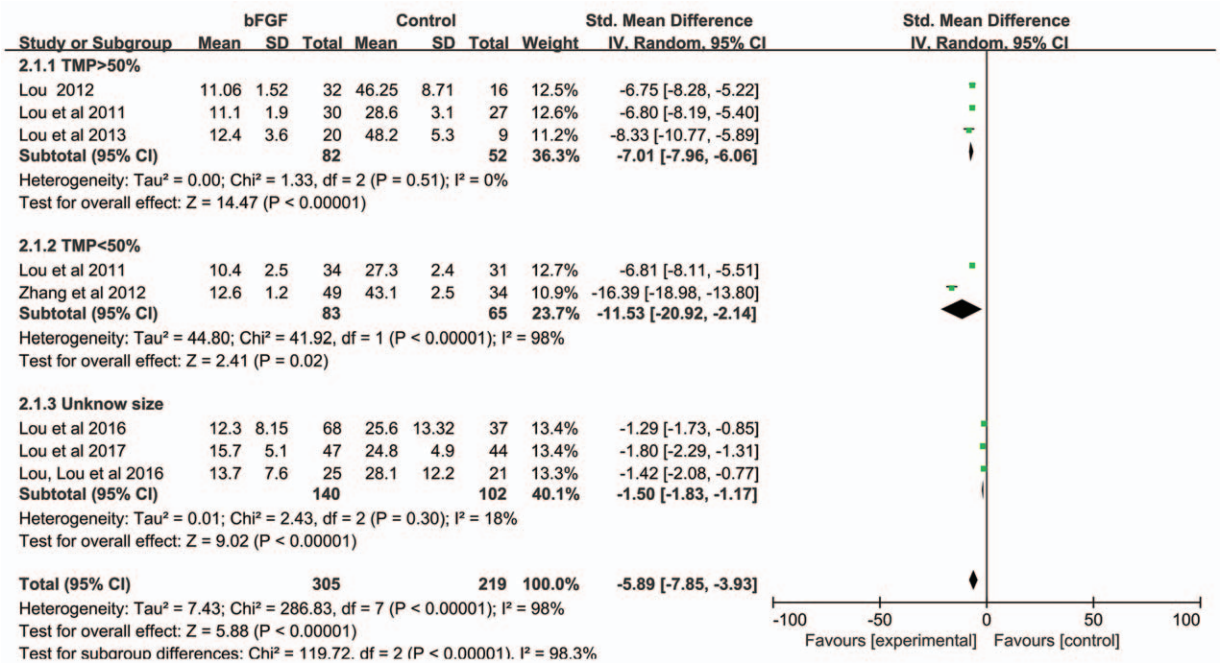

FIG. 4. Forest plot of healing time between bFGF and control group. Subgroup analysis was performed in (*A*) large perforation, (*B*) small perforation, and (*C*) unknown size. bFGF indicates basic fibroblast growth factor; CI, confidence interval; IV, inverse variance.

bFGF for TM repair. To our knowledge, this meta-analysis is the first of its kind.

Closure rate serves as the fundamental importance in the evaluation of TM regeneration (5,9). The success rates of bFGF application have been reported to be as high as 89.3 to 100% and 83 to 98.1% in acute and chronic TM perforations respectively (12). Our analysis supports previous findings that bFGF can significantly enhance the closure rate of TM perforations. Given that some clinical trials are non-controlled, we only included studies with control group for this current study. Closure rate in our review ranged from 89 to 100% with the application of bFGF. A meta-analysis of pooled results further demonstrated that bFGF has a statistically significant effect on closure rates of perforated TMs.

Similarly, our results support the current trends in the literature indicating that bFGF significantly shortens the perforation closure time (12). However, we could only include data for acute perforations. Based on our study, the SMD value for the closure time was −5.89, demonstrating a large and statistically significant effect size. This is clinically relevant, since earlier closure reduces morbidities, costs associated with follow up reviews and audiology, as well as quicker return to sporting activities and fewer precautions required to prevent water entering the ear canal. Lou et al. (12) reported that closure time was around 2 weeks in patients with traumatic TM perforation following bFGF treatment based on 11 clinical studies. In our study, subgroup analysis showed that those studies with small perforations (<50% of the TM) accounted for high heterogeneity, which may be due to the small sample size and insufficient trials.

To date, the underlying mechanism of bFGF in promoting the TM regeneration has not been fully understood (12). Previous studies have postulated the possible effects of bFGF on cell proliferation and migration of

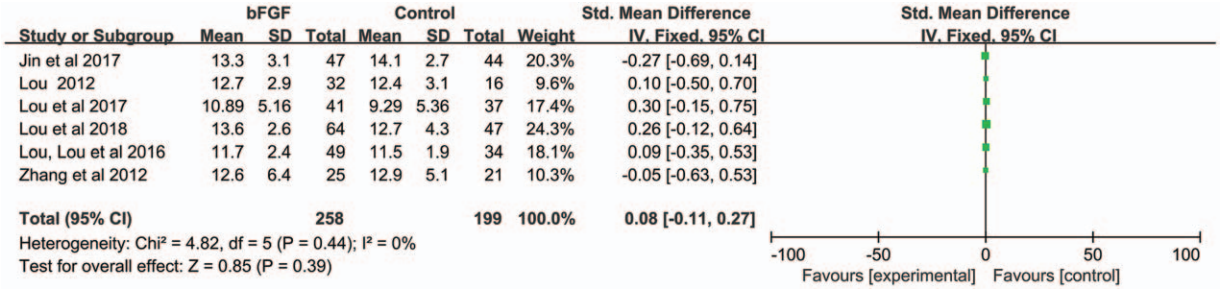

FIG. 5. Forest plot of hearing improvement between bFGF and control group. bFGF indicates basic fibroblast growth factor; CI, confidence interval; IV, inverse variance.

endothelial cells and fibroblasts and production of proteases in vitro (32,33). Studies have shown that spontaneously healed TMs tend to be thin and dimeric due to incomplete formation of the fibrous layer (34,35), resulting in higher risks of reperforation. It is thought that bFGF promotes neovascularization and improves the arrangement of collagen fibers in the fibrous layer of a TM (10), thereby reducing the risk of reperforation.

Despite an improvement in healing outcomes, this study did not show a statistically significant improvement in hearing compared with spontaneous healing. The SMD for the hearing gains was only 0.08, which is a change that is not clinically significant. The outcome of hearing function for TM repair depends on various factors, including graft type, surgical approach, Eustachian tube function, site and size of perforations (5,36). Our study did not show an improvement in hearing following bFGF treatment, suggesting that bFGF may not have improved sound conduction despite the probable improvement in TM structure. However, our results of hearing are limited to those with traumatic TM perforations. It should be noted that, compared with other causes of perforations, traumatic perforations tend to heal more spontaneously and rapidly (28,37). Moreover, it has been reported that there is no significant impact on hearing upon complete closure of the acute TM perforations (37) compared with pre-perforation hearing. Hence, these studies mainly showed faster hearing recovery following the bFGF treatment rather than improvement in hearing.

The use of exogenous bFGF in TM regeneration has been studied extensively (12). In the last decade, bFGF has been studied as an effective and convenient therapy with numerous experimental and clinical studies (12). The use of bFGF as an eardrop applied directly via transcanal route has been shown to be simple and convenient. Although a majority of studies reported encouraging outcomes, this is mainly in the setting of acute or traumatic TM perforation models. For chronic TM perforation, bFGF has been used in combination with a graft material such as Gelfoam (23,38,39) or a collagen patch (6,40), thus the direct effectiveness of bFGF in chronic perforation is unclear. In addition, the most effective dosage (12,41), duration and frequency of application (42), and presence of patching materials (43) have yet to be fully investigated (44). Mondain et al. (45) found that high doses of bFGF (e.g., 400 ng) caused myringitis; in contrast, Lou (19) indicated this dosage was not associated with any severe complications such as cholesteatoma and infection. Although otorrhoea was reported with excessive application of a single dosage, it only prolonged the closure time and did not affect the closure rate (12). The safety of bFGF has been investigated extensively in the literature (23,38,45). In addition, Lou et al. (12) reported that patching materials were not necessary for topical application of bFGF in acute TM perforation. However, other studies have shown that bFGF in combination with bioscaffolds can significantly promote the TM healing (6,23,38–40). Indeed, graft materials and bFGF enhance TM repair via different ways where the bioscaffolds offer structural support to guide the neo-tissue regeneration and bFGF facilitates cellar migration and proliferation (10,43).

There are several limitations in our study. Firstly, a major limitation is that only studies conducted in Japan and China fulfilled the inclusion criteria and were included in the study. In fact, a majority of the included studies was reported by Lou's group in China. Specifically, six randomized controlled trials (RCT) and four prospective studies among Lou's studies. Only one study published in 2011 is a retrospective study. It is unclear whether these patients included were individual or overlapped patients, which may lead to selection bias. If this represents overlapped patients, it would significantly skew the results of our analysis. Moreover, there may be potential biases due to ethnic and genetic differences among Asians as compared with other ethnic groups. Secondly, our analysis is based on only 14 clinical controlled studies. Though most of them were published recently, the sample size is relatively small. Thirdly, the majority of studies enrolled focused on the efficacy of bFGF in cases of acute or traumatic TM perforation. Only three studies evaluated the closure rate of bFGF in chronic TM perforation. These studies did not report healing time and hearing improvement, thus limiting our analysis. Therefore, well-designed RCTs studies, which focus on chronic TM perforation with larger sample populations without any patching materials are warranted.

CONCLUSION

This meta-analysis has identified that the application of bFGF can significantly enhance the closure rate as well as shorten healing time for the TM regeneration. There is no evidence, as assessed only from studies of acute perforations, to show that bFGF improves hearing. Given its ease, availability and safety, bFGF can be used effectively for the TM repair. Additional high-quality multi-center randomized controlled clinical trials are needed to further support its efficacy in different ethnic groups especially with chronic TM perforations.

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