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Topical recombinant human epidermal growth factor for diabetic foot ulcers: a meta-analysis of randomized controlled clinical trials

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Titles: Topical recombinant human epidermal growth factor for diabetic foot ulcers: a meta-analysis of randomized controlled clinical trials

Running Head: Meta-analysis of Topical rhEGF for DFU

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Abstract

Diabetic foot ulcer and its complications are becoming more and more serious problems threatening people's health. In the last decade, multiple growth factors and their combined applications have shown potentials in promoting the healing process of diabetic foot ulcers. The purpose of this study is to perform a meta-analysis of the efficacy and safety of topical recombinant human epidermal growth factor (rhEGF) on the treatment of diabetic foot ulcers. As of November 30, 2018, we had conducted a comprehensive review of Pubmed, EMBASE, Cochrane Library databases, and Web of Science. Seven randomized controlled trials (RCT) that involved 610 participants were included in this review. The pooled results showed that topical rhEGF could significantly promote the healing of diabetic foot ulcers (*RR* 1.54, 95% *CI* 1.30 to 1.83; I^2 = 18%). Topical application of rhEGF could promote ulceration healing of diabetic feet of Wagner grade 1 or 2 significantly (*RR* 1.61, 95% *CI* 1.32 to 1.97; I^2 = 0%), and intralesional injection of rhEGF appeared to promote the healing of more severe ulcers (*RR* 2.06, 95% *CI* 0.35 to 12.22; I^2 = 50%). However, patients developed more Shivering (*RR* 4.67, 95% *CI* 1.39 to 15.71; I^2 = 0%), Nauseas/Vomiting (*RR* 2.18, 95% *CI* 0.72 to 6.55; I^2 = 0%) in the group of intralesional injection of rhEGF compared with the control group, although these symptoms were not found with the topical application of rhEGF. No serious complications were found associated with topical rhEGF. Topical rhEGF treatment of diabetic foot ulcers has showed a broad application prospect, yet more relevant well-designed randomized controlled trials are needed in the future.

Key word:diabetic foot; chronic wound; EGF; rhEGF; Meta analysis.

Diabetic foot which refers to pathological changes caused by chronic diabetes mellitus[1] presents as wounds that extend below the ankle level and involve the entire skin layer[2]. Diabetic patients become prone to get foot ulcers for several reasons including abnormal sensory function of the foot skin combined with periodic repetitive stimulation，peripheral neuropathy and vascular disease. Diabetes mellitus with foot ulcer complications has become a more and more serious problem affecting the general population. According to the International Diabetes Federation (IDF), 415 million people worldwide had developed diabetes in 2015. At that time, the estimated global cost of diabetes was $1.3 trillion[3]. In developed countries, about 5% of the diabetics have foot problems, and consume 12% to 15% of the total health resources. In developing countries, the proportion of foot problems of those with diabetes is as high as 40%[4]. The foot problems usually have multiple complications, such as chronic rest pain, intermittent claudication, foot infections, osteomyelitis, and even amputation in some severe cases[4].

At present, the conventional treatments include infection control, wound care, debridement, revascularisation as requested, offloading, and using dressings that are conducive to wound healing, but the curative effect is not satisfactory. Even with comprehensive treatment, the cure rate is only 24 to 30 percent after 12 to 20 weeks. Amputation is still a serious threat to disability and can even result in death of

patients[5].

Several growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), peripheral blood mononuclear cells(PBMC) and their combined applications have shown potentials in promoting ulcer healing[6,7,8]. Wound healing can be divided into three stages: inflammation, proliferation and remodeling[9], which requires coordination and integration of delicate and complex biological events. The growth factors participating in those biological events work by stimulating chemotaxis, cell proliferation, extracellular matrix deposition, angiogenesis, and tissue reconstruction[10,11].

EGF was discovered in mouse salivary glands in 1962[12]. EGF, secreted by platelets, macrophages, mononuclear cells and fibroblasts, activates receptors to stimulate cell proliferation and wound healing. Local administration of EGF in the clinic began in 1989 to accelerate the healing process of various peripheral wounds. The process of topically applied EGF is not without problems and is not generally accepted for two reasons. The first one is related to the outcomes of clinical trials[13]. Some studies have shown that topically applied EGF has a limited effectiveness, because it can be degraded by proteases from the biofilm covering the lesion as well as from its exudate[14]. Another is the concern that EGF can promote the proliferation of malignant cells. Meanwhile, a large number of basic and clinical trials on its effectiveness and safety have been conducted, and many of them showed encouraging

results[15-17]. Several randomized controlled trials have assessed the curative effect of topical EGF on healing diabetic foot ulcers, but a systematic evaluation of their findings has not been conducted. Therefore, we have conducted a systematic review in order to evaluate the efficacy of topical epidermal growth factor on healing diabetic foot ulcers.

Methods

Eligibility criteria

Studies were included if: (1) The language was English; (2) Patients with diabetic foot ulcers were investigated; (3) Report of outcomes were included; (4) Comparisons of topical recombinant human epidermal growth factor (rhEGF) with placebo or conventional therapy were made; (5) The study designs were Randomized controlled Clinical Trials (RCTs).

Studies were excluded if: (1) The literature had no required results; (2) There was no placebo or conventional group in the study; (3) The study was a repeated one by the same author or team.

Information sources and search strategy

Two reviewers searched the Pubmed, EMBASE, Cochrane Library databases, and Web of Science independently and comprehensively. The language was limited to

English, and the final search was performed on November 30, 2018. Before formulating the retrieval strategy, we conducted multiple pre-retrievals to have better search results. We used the following search terms: (1) diabetic foot ulcer, diabetic foot, diabetic ulcer, diabetic wound, and DFU, and (2) epidermal growth factor, EGF, rhEGF. In addition, we reviewed all references of the relevant articles.

Study selection

The two researchers used Endnote X7 software to manage the studies. We conducted preliminary screening of titles and abstracts independently to exclude studies that did not meet the inclusion criteria. Then we read the full text of the preliminarily selected articles carefully to finalize the eligible literature. Differences were resolved by joint discussions with the third author.

Data collection

We made a table for literature data extraction in advance. Then we read the full text and filled in the form carefully. Data regarding the publication date, first author, country, number of participants, characteristics of the participants, details of the topical rhEGF therapy, treatments and follow-up time, number of ulcers healed and other evaluation parameters, and the incidence of adverse events were recorded. We contacted the author for the data required in graphs if it was not described in the article. In the case of no response, the graph was measured by GetData Graph Digitizer software to obtain the data. However, the accuracy of the data obtained this

132 way is regarded low.

133

134 Statistical analysis

135 RevMan 5.3 software was used to perform the analysis. We presented dichotomous
136 outcomes as risk ratios (*RR*s) with their corresponding 95% *CI*s. For continuous
137 outcomes, we used mean differences (*MD*) with their 95% *CI*s as the measure of
138 treatment effects. I^2 was used to evaluate interstudy heterogeneity. A I^2 value higher
139 than 50% was considered to have statistically significant heterogeneity[18]. If there
140 was homogeneity between studies, we used a fixed effects model for analysis. If the
141 studies were obviously heterogeneous, the random effect model or subgroup analysis
142 was adopted after analyzing the sources of heterogeneity.

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145 Results

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147 Study selection

148 The initial literature search included a total of 336 articles. After careful screening
149 of abstracts and full texts, seven randomized controlled studies[19-25] were finally
150 included. All the studies included were published as journal articles. The literature
151 screening process is shown in Figure 1.

152

153 Characteristics of eligible studies

The seven studies involved a total of 610 participants, 347 in the experimental group and 263 in the control group. The total number of patients in each of the studies ranged from 34 to 167. These studies were published between 2003 and 2018. Most of the studies came from Asia, except one from Mexico and another one from Cuba. The average age of the participants ranged from 55 to 69. Follow-up duration of most studies ranged from 4 to 12 weeks with the exception of one study whose patients were followed up for one year[21]. rhEGF was administered in five studies by topical application and two studies by intralesional injection. Severe ischemic ulcers were excluded in all studies and all studies described wound care, debridement, and infection control for ulcers prior to treatment.

The characteristics of included studies are shown in Table 1, and the summary of participants is presented in Table 2.

Quality assessment

The risk of bias was assessed by the Cochrane assessment tool (Figure 2), and the quality of the studies ranged from low to high. All the included studies were described as randomized clinical trials, and four studies[19,20,22,25] had detailed randomization methods such as using random number tables, internet-based systems or envelope. Four studies[19,22,23,25] reported the allocation procedure. Five studies[19,22-25] claimed to be double-blinded, one[25] of which did not report details. Three studies[19,22,23] described the details of loss to follow-up and all randomized patients of them were

included in the data analysis. One study[23] might have other biases, because its grouping was partially disrupted due to ethical issues after 2 weeks of treatment.

Effect of topical epidermal growth factor on diabetic foot ulcer healing

Six studies[19,21-25] with a total of 610 participants contributed to evaluate the proportion of wounds completely healed during follow-up. We pooled the six studies with a fixed□effect model. Meta□analysis indicated that the topical rhEGF group had a higher proportion of wounds completely healed during follow□up compared with the control group (*RR* 1.54, 95% *CI* 1.30 to 1.83; I^2 = 18%) (Figure 3).

The duration of treatment for these studies was 4 weeks, 8 weeks, 12 weeks respectively. In order to determine the effect of treatment time on efficacy, a subgroup analysis was performed. A random-effect model indicated that the rhEGF group showed higher complete healing than the control group regardless of the treatment duration of 4 weeks (*RR* 2.33, 95% *CI* 0.54 to 10.11), 8 weeks (*RR* 1.67, 95% *CI* 0.97 to 2.86; I^2 = 61%) or 12 weeks (*RR* 1.50, 95% *CI* 1.20 to 1.88; I^2 = 0%) (Figure 4). However, the quality of the evidence was low due to small sample size and moderate statistical heterogeneity.

We also performed a subgroup analysis of rhEGF administration methods. A random-effect model indicated that the rhEGF group had a higher proportion of wounds completely healed by topical application (*RR* 1.61, 95% *CI* 1.32 to 1.97; I^2 = 0%) or intralesional injection (*RR* 2.06, 95% *CI* 0.35 to 12.22; I^2 = 50%) (Figure 5). What is worth mentioning is that all studies in the topical application subgroup

included diabetic foot ulcer of Wagner grade of 1 or 2, while those in the injection subgroup included more severe ulcers. Again, the quality of the evidence was low due to unclear risk of bias in the original trial and moderate statistical heterogeneity.

Two studies[19,22] reported that the average area of the ulcer decreased after treatment and four[19,20,23,25] studies reported the ulcer healing time (table 2). We did not perform a test for the difference as different measure terms were used and high heterogeneity between studies was present.

Sensitivity analysis and publication bias

Sensitivity analysis included 6 studies[19,21-25] and did not identify any significant change in the findings. The funnel plot was not used to assess publication bias because the Cochrane handbook deemed it inappropriate due to the small number of studies included[24].

Adverse events

Five studies[19,21-23,25] mentioned adverse events in the results, such as pain, infection, cellulitis, osteomyelitis and amputation. Three of the studies[21,23,25] recorded the number of amputations, but none described the details of limb salvage, such as through bypass, endoluminal technique or other techniques. There was no evidence that these adverse events were associated with topical rhEGF. Meta□analysis indicated that shivering (*RR* 4.67, 95% *CI* 1.39 to 15.71; I^2 = 0%) and nauseas/vomits (*RR* 2.18, 95% *CI* 0.72 to 6.55; I^2 = 0%) occurred more often in the

topical rhEGF group compared with the control group (Figure 6,7). It's worth mentioning that intralesional injection of rhEGF was reported in all those cases. Fernandez-montequin JI's study[23] reported a higher number of adverse events than others possibly because it included higher grade of ulcers. There was no significant difference in the incidence of other adverse events between the treatment group and the control group (Table 3).

Discussion

We performed the meta-analysis to identify the efficacy and safety of topical rhEGF for diabetic foot ulcer. A total of seven studies involving 610 participants were included. The results indicated that topical epidermal growth factor could improve the healing of chronic ulcers of the diabetic foot patients, showing a higher rate of complete ulcer healing. The results were relatively robust, as sensitivity analysis had shown that deletion of any study would not change the direction of the outcomes. At the same time, topical rhEGF seemed to be safe, because there was no difference in the proportion of serious complications. Although the percentage of people who developed shivering and nauseas/vomits was higher, these side effects were described as mild, which might be related to the way intralesional injection was administered.

Wound healing requiring an orchestrated integration of complex biological events including cell migration, cell proliferation, angiogenesis and tissue integrity

repair[27,28] is a delicate and complex process. Growth factors play an important role in the process. When the skin barrier is broken and the cells around the wound are exposed to warning signals, growth factors act as soluble messengers to establish communication networks between different cell groups and extracellular matrix, precisely inducing and regulating the healing response. Frustration at any step in this process such as defective fibroblast activity, poor angiogenesis, blocked cell migration and decreased local growth factor activity can lead to delayed wound healing [29,30]. Diabetic foot ulcer is a type of refractory wound with specific and distinctive risk factors. The main etiological factors for it are that vascular endothelial cytotoxicity caused by hyperglycemia leads to dysfunction of microcirculation, and then the resulting hypoxia leads to a series of pathological cellular and molecular changes that eventually show a bad outcome.

Epidermal growth factor is a 6 kDa protein secreted by platelets, macrophages, monocytes and fibroblasts. EGF activates mesenchymal cells and epithelial cells, and stimulates angiogenesis and epidermal repair after injury by acting in an autocrine and paracrine manner on the corresponding receptors[31,32]. The efficacy of EGF in the healing of acute and chronic wounds is different. In vitro studies have shown that EGF is up-regulated around the wound after acute injury, and epithelialization and wound tensile strength is enhanced[33], while EGF and its receptors are down-regulated in chronic wounds with delayed wound repair. This may be due to the increased levels of inflammatory cytokines and metalloproteinases in chronic wounds, which lead to the destruction of growth factors and thus obstruction of the

transmission pathway[34,35]. As a result, the clinical efficacy of topical EGF for chronic wounds was not satisfactory initially[13]. But enthusiasm has not waned, and a large number of clinical trials has been going on. Our meta-analysis showed positive results, perhaps with the reasons as follows: (1) Most studies included patients with less severe diabetic foot ulcers, and in addition thorough debridement and antibiotic treatment before topical EGF was applied cleared most necrotic tissues, bacteria and inflammatory factors. (2) Local EGF at high concentrations allowed sufficient amounts of exogenous growth factors to enter the necrotic tissue and played a role. (3) Although EGF was degraded rapidly after entering tissues, cells activated by stimulation continued to coordinate the healing response.

It is still a research topic how to make topical EGF overcome the adverse effects of the microenvironment of chronic wounds and exert its effectiveness. It can be several clinical research directions for topical EGF to be applied in combination with bioactive dressings[36], multiple growth factors[37], tissue engineering vectors and slow-release systems. Treatment of diabetic foot ulcers by intralesional injection has also been used to increase the efficiency of EGF and showed positive results[22,23]. We performed a subgroup analysis and the results showed that topical administration of EGF could achieve better clinical efficacy in ulcer healing by both ways of topical application and intralesional injection. We hypothesize that intralesional injection may be more appropriate for higher grade ulcers, because it looks that the more severe the ulcer was, the lower the efficacy of topical EGF and intralesional injection was in playing a role in overcoming local constraints. In our analysis, the two studies in the

intralesional injection group also had higher grade ulcers than the topical application studies. Another possible reason why patients were less receptive to the method of intralesional injection than topical application, was that it could cause pain in the injection site and had other side effects. However, there are no randomized controlled studies comparing the two methods treating diabetic foot ulcers right now, and more evidence is needed in the future.

The safety of clinical application of topical EGF is another focus. Our statistical analysis has not shown any significant difference in the incidence of adverse events between the treatment group and the control group, except that shivering and nauseas/vomits occurred more frequently in the treatment group. However, these adverse events should not be exaggerated because they were described as mild and easily manageable[22,23], consistent with previous reports[38,39]. Another major concern of exogenous EGF use is that it could promote the development of neoplasia, but it was not observed in any of the subjects. However, the follow-up time was too short of all included studies for this purpose. More basic and clinical trials with well-designed and longer follow-up time are needed.

The limitations of this study are as follows: (1) The quality of some included literatures was low. Although the authors reported that their studies were randomized, the random sequences and blind details were not described in the original articles. (2) The number of RCTs included was small, leading to the inability to evaluate some indicators and limiting the analysis of publication bias. (3) There were differences in dressing types, offloading devices, baseline ulcer size and treatment frequency, which

resulted in the possibility of heterogeneity. (4) One study opened the trial after two weeks of treatment because of the constraints imposed by the Ethics Committees. Even with the methodological treatment, biases might have still existeded. (5) Although all studies reported exclusions of severe ischemic ulcers, the degree of severity was described variably without the specifics about the vascularistaion of the leg. (6) The origin of the works did not correspond to a homogeneous recruitment.

Conclusion

Compared to standard therapies, topical recombinant human epidermal growth factor could help accelerate the healing of diabetic foot ulcers at 4-12 weeks of treatment. Topical application of rhEGF could improve ulceration healing significantly in diabetic feet of Wagner grade 1 or 2, while intralesional injection of rhEGF might be effective for more severe ulcers. The majority of side effects were mild and easily manageable, and no significant adverse events associated with local use of rhEGF were reported. More well-designed clinical trials with long follow-up time are required to further examine the topical rhEGF therapy in management of diabetic foot ulcer in the future.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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Table 1. Characteristics of included studies.

Abbreviations: RCT, randomized controlled trial; NA, not available.

462 Table 2. Summary of participants in included studies.

463 Abbreviations: rhEGF, recombinant human epidermal growth factor; No., Number;

464 DM, diabetes mellitus; NA, not available.

465

466 Table 3. Summary of Adverse Events.

467 Abbreviations: rhEGF, recombinant human epidermal growth factor; No., Number.

468

469 Figure 1 Study flow diagram.

470

471 Figure 2 Summary of risk of bias of the included studies.

472

473 Figure 3 Forest plots and meta-analysis of complete healing rate.

474 M-H, Mantel-Haenszel method; CI, confidence interval.

475

476 Figure 4 Forest plots and meta-analysis of complete healing rate and interventions

477 by treatment duration.

478 M-H, Mantel-Haenszel method; CI, confidence interval.

479

480 Figure 5 Forest plots and meta-analysis of complete healing rate and interventions

481 by rhEGF administration methods.

482 M-H, Mantel-Haenszel method; CI, confidence interval.

483

Figure 6 Forest plots and meta-analysis of the incidence of Shivering.

M-H, Mantel-Haenszel method; CI, confidence interval.

Figure 7 Forest plots and meta-analysis of the incidence of Nauseas/Vomits.

M-H, Mantel-Haenszel method; CI, confidence interval.

Table 1. Characteristics of included studies.

Author, year	Country	Study design	Multicenter trial	Type of diabetes	Concentration	Administration of rhEGF	Frequency	Treatment time	Wagner grade
Park KH, 2018[19]	South Korea	RCT	Yes	Ⅰor Ⅱ	50 μg/ml	Topical application	2 times/day	12 weeks	1 or 2
Xu J, 2018[20]	China	RCT	No	Ⅱ	40iu/cm2	Topical application	1 times/day	60 days	2
Singla S, 2014[21]	India	RCT	No	Ⅰor Ⅱ	NA	Topical application	NA	8 weeks	1 or 2
Gomez-Villa R , 2014[22]	Mexico	RCT	Yes	Ⅰor Ⅱ	75 μg/ml	Intralesional injection	3 times/week	8 weeks	1,2 or 3
Fernández-Montequín JI, 2009[23]	Cuba	RCT	Yes	Ⅰor Ⅱ	75 μg/ml 25 μg/ml	Intralesional injection	3 times/week	8 weeks	3 or 4
Afshari M, 2005[24]	Iran	RCT	No	Ⅰor Ⅱ	NA	Topical application	1 times/day	4 weeks	1 or 2
Tsang MW, 2003[25]	Hong Kong, China	RCT	No	Ⅰor Ⅱ	0.04% 0.02%	Topical application	NA	12 weeks	1 or 2

Abbreviations: RCT, randomized controlled trial; NA, not available.

Table 2. Summary of participants in included studies.

Author, year	Groups	No. of Patients	Age, Years	Male	Ulcer duration (weeks)	Ulcer baseline (cm^2)	DM duration (years)	HbA1c	Ulcer reduces area	Complete healing time	Complete healing rate (%)
Park KH, 2018[19]	rhEGF	82	56.52 ± 12.71	55	41.23 ± 75.26	2.80 ± 3.72	NA	7.87 ± 1.46	2.47 ± 3.53	56 days	60 (73.2%)
	Control	85	59.31 ± 12.64	49	31.71 ±64.5	2.35 ± 2.69	NA	7.89 ± 1.73	1.75 ± 2.91	84 days	43 (50.6%)
Xu J, 2018[20]	rhEGF	50	65 ± 3.65	25	16 ± 0.62	4.7 ± 0.3	13 ± 4.88	NA	NA	38.51 ±1.46 days	NA
	Control	49	63 ± 4.56	25	13 ± 0.35	4.2 ± 0.4	12 ± 4.26	NA	NA	47.52 ±1.82 days	NA
Singla S, 2014[21]	rhEGF	25	58.8	21	NA	19.56	NA	NA	NA	NA	23 (92.0%)
	Control	25	55.84	23	NA	21.2	NA	NA	NA	NA	11 (44.0%)
Gomez-Villa R, 2014[22]	rhEGF	17	62.1 ± 12.8	9	25.8 ± 44.0	19.2 ± 15.7	17.3 ± 10.0	NA	12.5 ± 1.58	NA	4 (23.5%)
	Control	17	55.1 ± 10.6	12	36.5 ± 75.8	11.9 ± 11.8	15.3 ± 8.4	NA	5.2 ± 0.80	NA	0 (0%)
Fernández-Montequín JI, 2009[23]	75 μg rhEGF	53	63	28	4.3	28.5	19.5	NA	NA	14 weeks	40 (75.5%)
	25 μg rhEGF	48	65.5	21	4.3	20.1	15	NA	NA	12 weeks	25 (52.1%)
	Control	48	64	27	4.9	21.8	15	NA	NA	20 weeks	25 (52.1%)
Afshari M, 2005[24]	rhEGF	30	56.9 ± 12.7	16	6.13 ± 5.49	87.5 ± 103.2	12.6 ± 7.5	10.5 ± 2.6	NA	NA	7 (23.3%)
	Control	20	59.7 ± 12.3	11	8.53 ± 7.93	103.4 ± 147.8	14.9 ± 7.1	10.9 ± 1.65	NA	NA	2 (10%)
Tsang MW, 2003[25]	0.04% rhEGF	21	62.24 ± 13.68	6	11.48 ± 14.68	3.40 ± 1.1	9.05 ± 6.19	8.5 ± 1.34	NA	6 ± 1 weeks	20 (95.2%)

0.02% rhEGF	21	64.37 ± 11.67	13	8.24 ± 5.55	2.78 ± 0.82	9.85 ± 7.79	8.69 ± 1.99	NA	NA	12 (57.1%)
Control	19	68.76 ± 10.45	10	12.00 ± 15.47	3.48 ± 0.82	10.11 ± 8.29	7.97 ± 1.81	NA	NA	8 (42.1%)

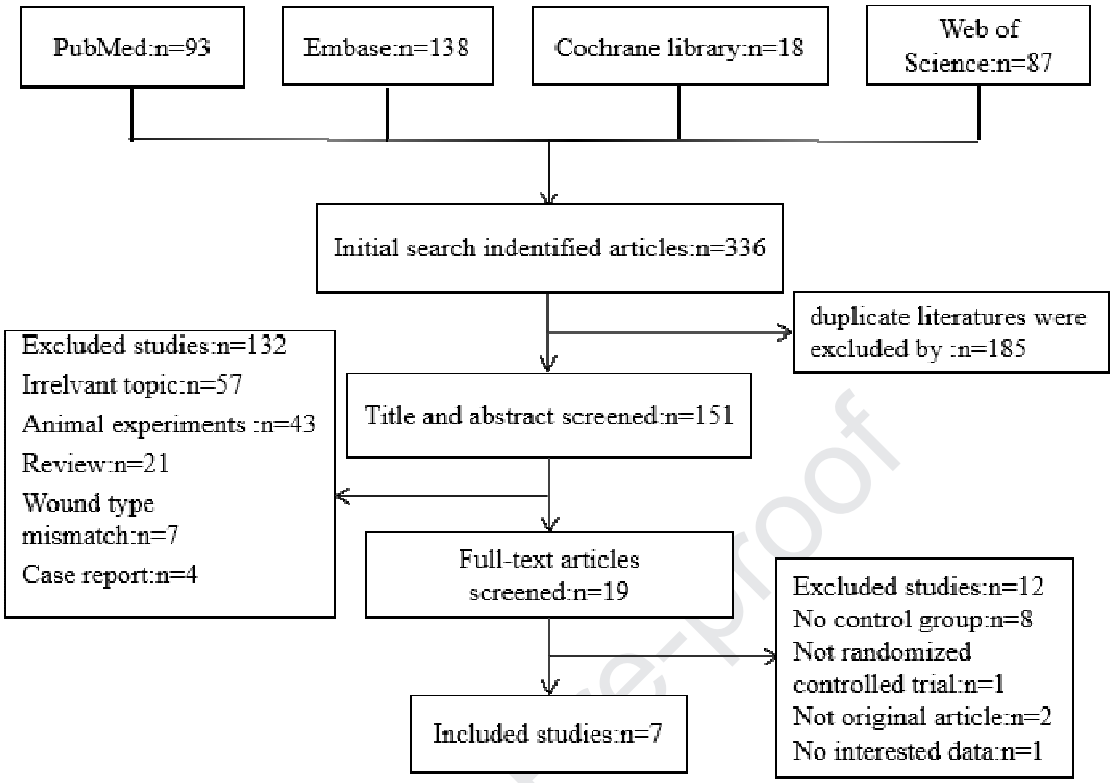
Abbreviations: rhEGF, recombinant human epidermal growth factor; No., Number; DM, diabetes mellitus; NA, not available.

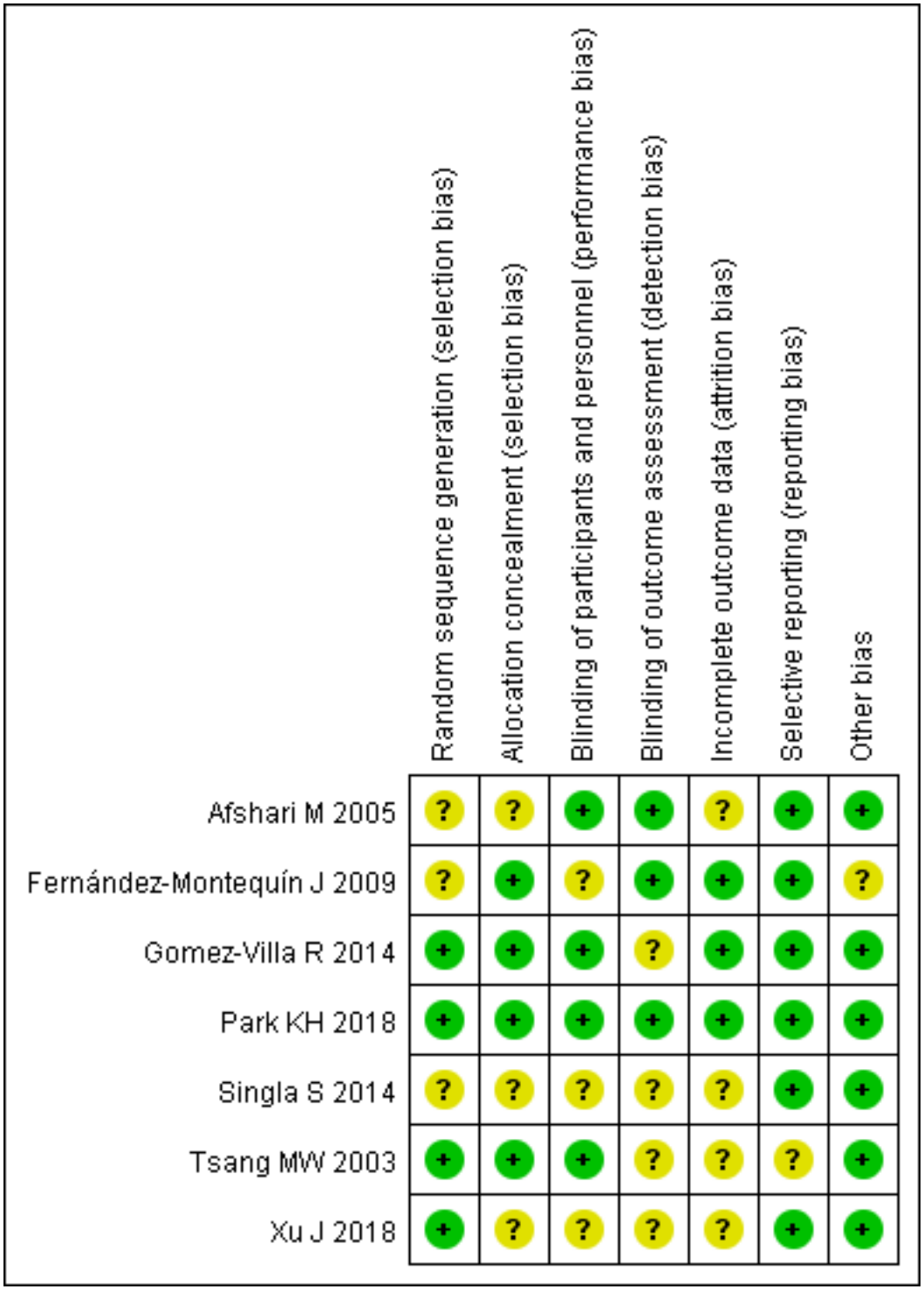
Table 3. Summary of Adverse Events.

Author, year	Administration of rhEGF	Groups	No. of Patients	Shivering	Nauseas/Vomits	Pain	Infection	Cellulitis	Osteomyelitis	Amputation	Death
Park KH, 2018[19]	Topical application	rhEGF	82	-	-	-	1	1	0	0	-
		Control	85	-	-	-	3	1	0	0	-
Singla S, 2014[21]	Topical application	rhEGF	25	-	-	-	-	1	-	1	-
		Control	25	-	-	-	-	2	-	0	-
Gomez-Villa R , 2014[22]	Intralesional injection	rhEGF	17	6	3	14	-	-	-	-	-
		Control	17	2	0	16	-	-	-	-	-
Fernández-Montequín JI, 2009[23]	Intralesional injection	75 μg rhEGF	53	11	7	13	7	-	-	7	2
		25 μg rhEGF	48	4	3	13	8	-	-	10	2
		Control	48	1	3	20	9	-	-	12	2
Tsang MW, 2003[25]	Topical application	0.04% rhEGF	21	-		-	-	-	0	0	-
		0.02% rhEGF	21	-		-	-	-	1	2	-
		Control	19	-		-	-	-	1	2	-

Abbreviations: rhEGF, recombinant human epidermal growth factor; No., Number.

Figure 1 Study flow diagram.

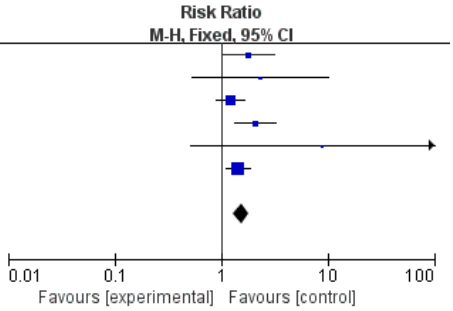


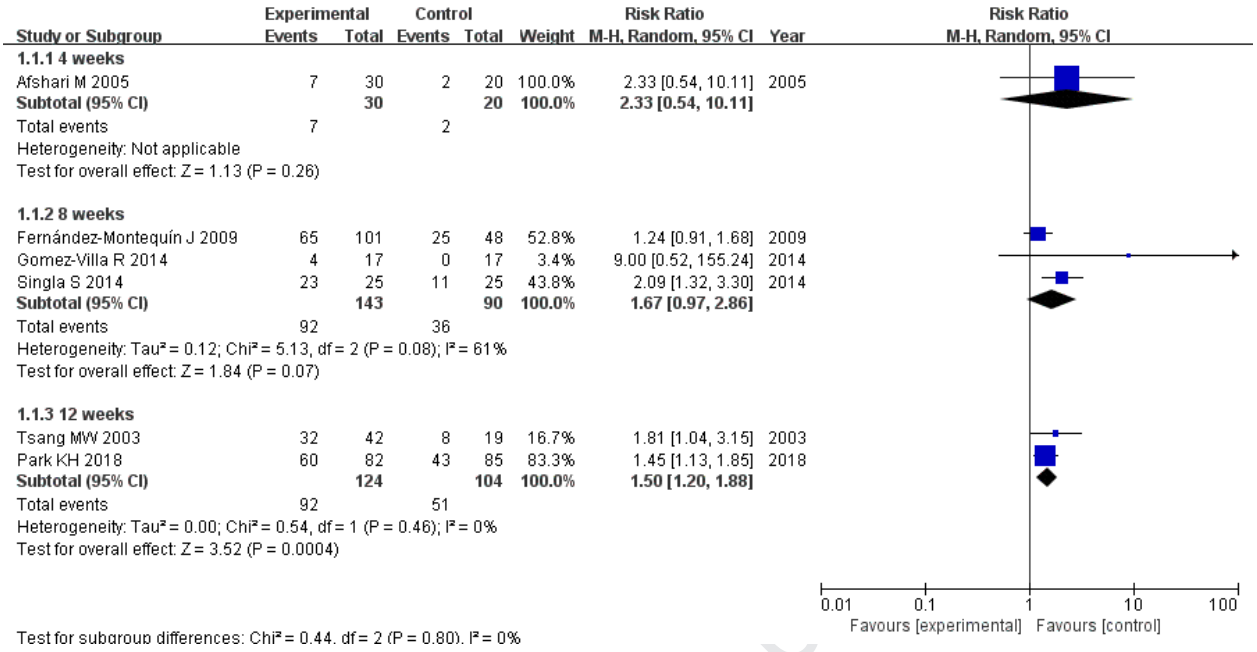

Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other bias
Afshari M 2005
Fernández-Montequín J 2009
Gomez-Villa R 2014
Park KH 2018
Singla S 2014
Tsang MW 2003
Xu J 2018

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Tsang MW 2003	32	42	8	19	10.9%	1.81 [1.04, 3.15]	2003
Afshari M 2005	7	30	2	20	2.4%	2.33 [0.54, 10.11]	2005
Fernández-Montequín J 2009	65	101	25	48	33.5%	1.24 [0.91, 1.68]	2009
Singla S 2014	23	25	11	25	10.9%	2.09 [1.32, 3.30]	2014
Gomez-Villa R 2014	4	17	0	17	0.5%	9.00 [0.52, 155.24]	2014
Park KH 2018	60	82	43	85	41.8%	1.45 [1.13, 1.85]	2018
Total (95% CI)		**297**		**214**	**100.0%**	**1.54 [1.30, 1.83]**	
Total events	191		89				

Heterogeneity: $Chi^2 = 6.06$, $df = 5$ ($P = 0.30$); $I^2 = 18\%$

Test for overall effect: $Z = 4.98$ ($P < 0.00001$)





Test for subgroup differences: Chi² = 0.44, df = 2 (P = 0.80), I² = 0%