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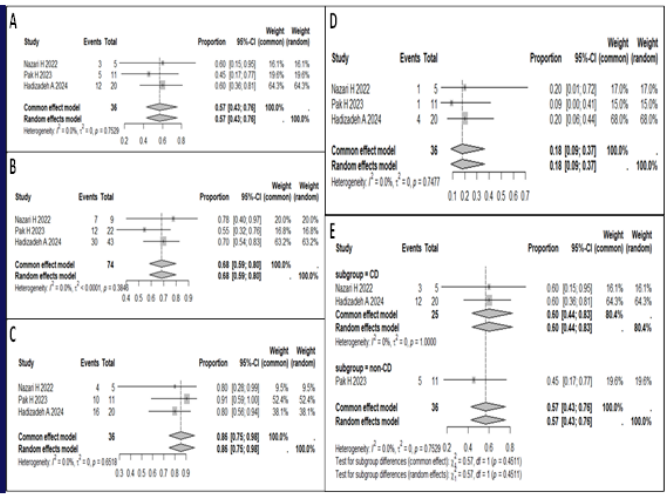
（1）The analysis of three studies included in this study suggested that MSC-Exos were effective in achieving complete healing in 57% of patients with complex PFs (95% confidence interval (CI) 0.43, 0.76).

（2）The pooled data further indicated that MSC-Exos could achieve complete healing in 68% of complex fistula tracts (95% CI 0.59, 0.80).

（3）Pooled analysis also showed that 86% (95% CI 0.75,0.98) of patients with complex PFs achieved clinical response post treatment, whereas 18% (95% CI 0.09, 0.37) demonstrated no clinical response after MSC-Exos treatment.

（4）Subgroup analysis revealed a higher healing rate (HR) in patients with Crohn's disease (CD) compared to those without CD (60% vs. 57%, respectively), with no statistically significant differences (P > 0.05).

（5）Notably, neither systemic nor local adverse effects have been reported in any study.



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Mesenchymal stem cell-derived exosomes for complex perianal fistula – A systematic review and single-arm meta-analysis

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Author´s contributions:

FC and XC reviewed the literatures. FC and XC collected and analyzed the data. CF and YL designed and supervised the study. All authors read and approved the final manuscript.

Conflict of Interest：

The authors declare that they have no conflict of interest.

Abbreviations list: PFs: perianal fistulas; MSC-Exos: mesenchymal stem cell derived exosomes; CI: confidence interval; HR: healing rate; CD: Crohn's disease; MSCs: mesenchymal stem cells; RCTs: randomized controlled trials; AEs: adverse events; EUA: examination under anesthesia; EUS: endoscopic ultrasonography; MRI: magnetic resonance imaging;

Abstract:

Objectives: We performed a single-arm meta-analysis to evaluate the efficacy and safety of MSC-Exos in the treatment of complex PFs.

Methods: This systematic review followed PRISMA guidelines and included studies published up to March 20, 2025. We searched four databases for studies evaluating efficacy and/or safety of MSC-Exos for complex PFs. Analyses were performed using R statistical software version 4.4.0.

Results: The analysis of three studies included in this study suggested that MSC-Exos were effective in achieving complete healing in 57% of patients with complex PFs (95% confidence interval (CI) 0.43, 0.76). The pooled data further indicated that MSC-Exos could achieve complete healing in 68% of complex fistula tracts (95% CI 0.59, 0.80). Pooled analysis also showed that 86% (95% CI 0.75,0.98) of patients with complex PFs achieved clinical response post treatment, whereas 18% (95% CI 0.09, 0.37) demonstrated no clinical response after MSC-Exos treatment. Subgroup analysis revealed a higher healing rate (HR) in patients with Crohn's disease (CD)

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compared to those without CD (60% vs. 57%, respectively), with no statistically significant differences (P > 0.05). Notably, neither systemic nor local adverse effects have been reported in any study.

Conclusion: This meta-analysis highlights the efficacy and safety of local administration of MSC-Exos in patients with complex PFs, offering valuable evidence to guide future clinical practice.

Keywords: Complex perianal fistula. Mesenchymal stem cells. Exosomes. Single arm. Meta-analysis. Systematic review.

Introduction

Perianal fistula (PF) is an abnormal tract connecting the anal canal to the skin, characterized by ulcerations with draining fistulous tracts around the anal region. It is a common anorectal disease and the incidence rate is second only to hemorrhoid, estimated to occur in 12.3 per 100,000 men and 8.6 per 100,000 women [1]. The pathogenesis of PF, an immune-mediated chronic recurrent disease, remains unknown, though it may be associated with cryptoglandular infection in most cases. Notably, 28% of patients with PF develop anal canal carcinoma within 20 years of their initial diagnosis [2]. The clinical manifestations of PFs include perianal cellulitis, perineal pain, purulent discharges, and bowel incontinence. Based on the fistula's course, the scope of lesions, and the number of external openings, PFs are categorised as simple or complex. The American Gastroenterological Association defines complex PFs as those that involve the upper part of the sphincter complex, are associated with pain or fluctuation suggesting a perianal abscess, and/or are associated with a rectovaginal fistula or anorectal stricture [3]. Complex PFs result in greatly diminished quality of life, and up to 59% of patients are at risk of faecal incontinence [4]. The primary objectives of complex PFs treatment encompass achieving fistula closure, preserving the integrity of the anal sphincter, preventing recurrence, and sustaining or improving the patient's quality of life. At present, treatments include combinations of medical interventions, surgical interventions, and mesenchymal stem cells (MSCs). Despite significant advancements in surgical and medical treatment methods, high proportions of patients experience lack of or inadequate response to treatment. Consequently, complex PFs pose substantial clinical and humanistic burden. The high failure rate of the current approach can be attributed to multiple factors, including the complex pathophysiology, bacterial overgrowth, infection, and underlying inflammation. These factors pose a serious barrier to effective mucosal healing. Considering the complexity of PFs, the challenging nature of their treatment, and the high recurrence rate in these patients, treatment with more potent and biocompatible methods is essential.

In recent years, tremendous progress has been made in the field of regenerative medicine

and stem-cell biology. MSCs, which are non-hematopoietic, multipotent adult stem cells, can be isolated from various adult tissues, such as the bone marrow, umbilical cord, adipose tissue, and peripheral blood [5]. They are able to down-regulate immune responses, exhibit anti-inflammatory properties, and promote tissue healing [6]. There are pieces of evidence that MSC-treatment has shown an encouraging success rate when applied to complex PFs [7]. Previously, it was widely held that the protective effects of MSCs were exerted through their migration to damaged tissues, engrafting, and interactions with other cells following infusion. However, an increasing number of studies currently indicate that the well-established roles of MSCs in immunomodulation and promotion of tissue repair may be mediated by paracrine mechanisms involving secretory factors, such as exosomes [8-9]. Exosomes, which are secreted by cells, are classified as a subtype of extracellular vesicles. These minute vesicles typically exhibit a diameter ranging from 30 to 150 nm and possess a density between 1.13 and 1.19 g/ml [10]. Exosomes carry a multitude of signaling moieties, including proteins, lipids, cell surface receptors, enzymes, cytokines, transcription factors, and nucleic acids and thus have a significant role in cell-cell communication [11]. MSCs secrete more exosomes than other cells [12]. Several studies have demonstrated that mesenchymal stem cell-derived exosomes (MSC-Exos) play a role in easing many inflammatory and autoimmune diseases [13]. Additionally, they are capable of reproducing MSC function and overcoming the limitations of traditional cell therapy. Besides, some studies showed the role of exosomes in the regeneration of the gastrointestinal tract and the immunomodulatory and proliferative properties of MSC-Exos[14-16]. So, these exosomes may be safe and effective treatment options for complex PFs. Moreover, concerns about stem cell therapies, such as the ethics and safety of administration in human subjects and the risks of tumorigenicity and adverse immunologic effects from direct cell transplantation, have further encouraged the application of MSC-Exos. Thus, our study aimed to evaluate MSC-Exos' safety and efficacy in treating complex PFs.

2. Materials and methods

2.1 Data Source and Search Strategies.

This review followed the PRISMA guidelines published in 2020[17]. We searched PubMed, Embase, Web of Science, and the Cochrane Library for all relevant available studies published from inception until March 20, 2025. The search keywords were “mesenchymal stem/stromal cells”, “stem cell”, “stromal cell”, “perianal fistula”, “cryptoglandular perianal fistula”, “exosomes”. The search strategy included subject terms and free words. We also checked the reference lists of the screened full-text studies to identify other potentially eligible trials.

2.2. Study Selection.

The study design was structured around the selection of clinical trials to evaluate the MSC-Exos for the treatment of complex PFs. The inclusion criteria were applied: (1) human studies;(2) Randomized Controlled Trials (RCTs), single-arm trials and observational studies involving patients with complex PFs;(3) studies on local MSC-Exos for the treatment of complex PFs. Irrelevant studies were excluded based on the following criteria: (1) meeting minutes, review articles, study protocols, correspondence, basic and animal experiments; (2) duplicate studies from the same trials; (3) studies that did not involve the use of MSC-Exos; (4) studies without quantifiable outcome data.

2.3. Data Extraction and Quality Assessment.

Two investigators independently extracted data from eligible studies. Disagreements were resolved by discussion with a third investigator. These investigators extracted study data features, including (1) study characteristics (first author, publication year); (2) type of study;(3) MSCs type; (4) type of PFs (CD or non-CD); (5) age of participants; (6) outcome assessment; (7) intervention (the injection dosage and frequency of exosomes) ;(8) outcome index (such as complete healing, clinical response and non-response); (9) adverse events (AEs). As shown in Table1, the basic characteristics of the 3 identified studies are listed. Two reviewers independently evaluated the risk of bias in each study using the ROBINS-I tool for non-randomized trials [18]. Any disagreements were resolved through discussion.

2.4. Statistical Analysis and Publication bias.

The meta-package of R (version 4.4.0) was used for single-arm meta-analysis. Normality test results determined whether proportions should be analyzed using untransformed data or Freeman-Tukey double arcsine transformation, and whether confidence intervals should be calculated via the Jackson method. The Chi-square test ($P < 0.05$) and I^2 statistic (≥50% = substantial heterogeneity) evaluated statistical heterogeneity. Fixed-effects models were applied for I^2 <50%, otherwise random-effects models were used. A value of $P < 0.05$ was considered statistically significant. When subgroup data were sufficient, further subgroup analyses were conducted. Publication bias was evaluated not only through funnel plot visually but also through Egger's test and Begg's test.

3. Results

3.1. Literature Search and Quality Assessment.

The initial search identified 98 articles, of which three single arm studies were included in our meta-analysis [19-21]. Fig.1 depicts the detailed process of literature screening. Two of these used umbilical cord MSC-Exos [19、21] and one used human placenta MSC-Exos[20]. One study

included participants with non-CD-related fistulas [20] and two studies included participants with CD-related fistulas [19、21]. All studies employed magnetic resonance imaging (MRI) plus physical examination for fistula healing assessment. The three included studies had a moderate risk of bias. Details are in Tables 2. The potential publication bias is shown by a funnel plot. Finally, the funnel plot was considered roughly symmetric by inspection (Fig. 2A). Egger's test was also performed to detect publication bias for included studies (p = 0.1595) (Fig. 2B).

3.2. Meta-analysis

3.2.1. Analysis of Complete Healing Following MSC-Exos Therapy

Three studies evaluated the efficacy of MSC-Exos for the treatment of complex PFs. The primary endpoint was complete healing at 6 months (a physical examination plus MRI was employed to evaluate the therapeutic responses of patients). The fixed-effects pooled healing rate (HR) of patients with complex PFs was 57% post MSC-Exos treatment (95% CI 0.43, 0.76) (Fig. 3A). The pooled analysis also showed that the HR of complex PFs tracts was 68% post MSC-Exos treatment (95% CI 0.59, 0.80) (Fig. 3B).

3.2.2. Analysis of Clinical Response Outcomes Following MSC-Exos Therapy

In our study, the secondary endpoints included clinical response rate post MSC-Exos treatment. Three trials reported raw data on the clinical response of patients with complex PFs, and the fixed-effects pooled clinical response rate was 86% (95% CI 0.75, 0.98) (Fig. 3C).

3.2.3. Analysis of Non-Response Outcomes Following MSC-Exos Therapy

Complex PFs are the most challenging scenarios, posing significant management challenges and remaining a great clinical hurdle. Some patients with complex PFs often suffer from non-response or non-remission to conventional surgical and medical management, with high recurrence rates. In our study, the fixed-effects pooled non-response rate for complex PFs after MSC-Exos treatment was 18%. (95% CI 0.09,0.37) (Fig. 3D).

3.2.4. Subgroup analyses of different type of complex PFs (CD vs non-CD)

The anti-inflammatory and immunomodulatory activities of MSC-Exos provide a rationale for novel cell-free therapies for PFs. Growing evidence has shown that MSC-Exos can be a potential

candidate for treating complex PFs. Certain risk factors (e.g., type of fistula, complexity, refractoriness duration) may affect response rate. Two studies reported raw data on patients with complex PFs associated with CD. The pooled HR in these patients was 60%, compared to 57% reported in a separate study of patients with complex PFs not associated with CD. This difference was not statistically significant ($P > 0.05$) (Fig. 3E).

4. Discussion

MSCs are adult stem cells with self-renewal and multidirectional differentiation potential that may be obtained from bone marrow, adipose tissue, dental pulp, peripheral blood and other tissues. MSCs have powerful immunomodulatory and tissue repair capabilities, holding significant therapeutic potential in the treatment of PFs [22-23]. Experimental evidence has demonstrated that MSCs actively mediate tissue regeneration through three key mechanisms: homing capacity to damaged sites, paracrine signaling pathways, and direct cell-cell interactions. Many studies have also shown that MSCs have many drawbacks, such as the need for a consistent supply of cells with stable phenotype, high costs, and time delays for the generation and handling of these cells. Moreover, the risks of iatrogenic tumor formation, cellular rejection and infusional toxicity in MSC transplantation remain unresolved. Therefore, identifying a cell-free therapy to avoid the shortcomings of MSCs in regeneration and repair is worthy of further effort. Now, emerging evidence shows that the efficacy of MSC treatment results mainly from paracrine effects, rather than trans-differentiation and implantation of MSCs [24-25]. Subsequent studies indicated that MSC-Exos are extracellular vesicles produced by parental cells via the paracrine pathway, and they significantly enhance the therapeutic efficacy of MSCs by mediating intercellular communication during angiogenesis, tissue regeneration, and immunomodulation [26-27]. Compared to parent cells, exosomes are smaller and less complex, making them easier to produce and store, and devoid of viable cells, eliminating tumor risks [28]. Moreover, MSC-Exos are less immunogenic than parent cells and serve as drug-delivery vehicles. Currently, exosomes are emerging as a compelling alternative to cell-free therapies used in clinical trials for various diseases, such as heart, liver, brain, skin, GI tract, and others [29-30].

To our knowledge, this is the first systematic review and meta-analysis to evaluate the safety and efficacy of MSC-Exos therapy for complex PFs. In conclusion, our meta-analysis suggested that MSC-Exos treatment in patients with complex PFs was feasible and safe, and none of the patients developed acute or latent complications and reactions. Of note in our study is that patients who received MSC-Exos also showed significant improvement in quality of life. Although fistula closure is the primary goal of treatment, maintaining durable closure is equally critical not only as an indicator of long-term therapeutic efficacy but also due to the increased

surgical complexity associated with recurrent cases. However, achieving sustained healing in complex PFs continues to pose significant challenges. In our study, all included patients were followed for at least 6 months, and the proportion of patients with healed fistulas was determined at the 6-month post-operative evaluation. All included studies showed good mid-to-long-term healing of fistulas after MSC-exosomes treatment. Therefore, we believe that healing of complex PFs persisted after MSC-Exos treatment. Additionally, Lu et al. (2024) evaluated umbilical cord MSC-Exos for treating complex PFs in rats, demonstrating significant wound healing acceleration, favorable safety, and mechanism linked to HIF-1α/TGF-β/Smad pathway activation [31]. The study by Larabi A et al in 2020 also indicated that exosomes of small size can stimulate intestinal epithelial repair, inhibit intestinal epithelial cell apoptosis, and modulate colon inflammation [32]. Wang Y et al study showed that human umbilical cord MSC-Exos alleviates DSS-induced inflammatory bowel disease -related intestinal fibrosis by inhibiting profibrotic molecules and intestinal fibroblast proliferation and migration by decreasing ERK phosphorylation [33]. These available data confirm that MSC-Exos therapy provides an effective method for patients with complex PFs. Furthermore, our study suggested that MSC-Exos therapy appears to achieve higher HR for patients with CD than non-CD (60% vs 57%, respectively), with no statistically significant differences ($P > 0.05$). This result may be related to the different pathogenesis of the two conditions. Although the initial fistula stages may differ in patients with CD from those in patients with fistulas of cryptoglandular (non-CD) origin, we believe that once a tract is formed and the inflammation becomes chronic, both fistula types will respond equally well to administration of MSC-Exos. Interestingly, the study by Nazari et al. revealed that MSC-Exos treatment was ineffective for tracts with fibrosis surrounding the tract and the internal opening [19]. The authors speculated that this occurrence results from the inability of exosomes to penetrate fibrotic tissue. Consequently, this treatment method is unsuitable for chronic conditions with significant fibrosis. However, we do not concur with the author's viewpoint, as a growing body of research increasingly demonstrates the therapeutic efficacy of MSC-Exos in fibrotic diseases [34-35]. Given the limitations of the included studies, we cannot directly compare the therapeutic effects of MSCs and exosomes on complex perianal fistulas (PFs). However, the application of MSC-Exos in tissue engineering demonstrates significant advantages over stem cell-based therapies. Firstly, the utilization of MSC-Exos presents several advantages over traditional cell-based therapies, such as lower immunogenicity and ease of production, handling, and storage [36]. Secondly, employing MSC-Exos in tissue regeneration therapies circumvents challenges associated with MSCs-based treatments, such as low cell survival rates and poor engraftment [37]. Thirdly, by utilizing MSC-Exos as an off-the-shelf product, the need for a large number of cells for transplantation is significantly reduced. This approach also mitigates potential alterations in phenotypic characteristics and therapeutic efficacy that may

arise from prolonged in vitro expansion of MSCs prior to transplantation. Moreover, the time and cost associated with the expansion and maintenance of cultured stem cells can be substantially decreased, enabling the immediate availability of ready-to-use MSC-Exos therapies for patient treatment needs. Finally, due to their natural substance transport properties and excellent biocompatibility, exosomes can also be used as drug carriers to release a variety of substances [38]. Thus, this available evidence indicates that MSC-Exos are superior to parental stem cells in organ injury treatment and regeneration and may become an effective alternative to MSCs for clinical applications. In our study, the exosomes used were mainly from human umbilical cord-MSCs and human placenta -MSCs. The study by Börger V et al has proposed that the phenotype and function of MSC-Exos may vary depending on the source of MSCs[39]. The study by Kang IS et al indicated that exosomes extracted from adipose tissues have a better angiogenic capability than those extracted from the bone marrow [40]. Due to the limitations of our current research, we cannot obtain the most suitable source of MSC-derived exosomes. In the future, further investigation is needed in clinical studies in a disease-specific manner. Common modes of exosome infusion include intravenous, intraperitoneal, subcutaneous, and topical injections. The distribution of exosomes in the organs of the body is affected by the different methods of injection. So, different modes of injection of MSC-Exos affect the treatment outcome of complex PFs. In our study, all MSC-Exos were administered via local intraluminal injection through the fistula tracts. An advantage of using MSC-Exos to treat an anal fistula is that, because tract resection is not required, the treatment does not injure the anal sphincter. It is well known that for effective therapeutic outcomes, it is crucial that exosomes are distributed in high concentrations to the target organ. Administering MSC-Exos via local intraluminal injection enables the concentration of exosomes at the lesion site to reach the highest level. Due to the limitations of our included studies, it is not possible to compare the effects of exosomes transplanted via different routes of administration on PFs. Further studies with more patients are warranted to better clarify the most appropriate injection route for clinical translation of MSC-Exos.

As ethical and safety concerns regarding the use of MSCs continue to grow, the safety of MSC-Exos in this regard still warrants our attention. Our study indicated that no adverse effects were evidenced during either the exosome administration and the follow-up. The use of MSC-Exos circumvents the risk of direct stem cell therapy by introducing a cell-free option. First, in addition to possessing similar biological functions to those of parental MSCs, they exhibit lower immunogenicity. Second, the risk of tumor formation can be further reduced due to the absence of living cells present in the body. Moreover, MSC-Exos have the advantages of smaller size, and nanosized exosomes can transfer efficiently to specific tissues after administration without aggregation in the lung microvasculature, avoiding the possibility of a pulmonary embolism

caused by administrated cells [41]. However, we cannot entirely rule out the possibility that the safety concerns associated with the administration of MSCs are not unrelated to exosomes. It is necessary to evaluate the safety and efficacy of exosomes derived from MSCs by involving a larger number of participants and extending the follow-up duration.

This study is a single-arm systemic meta-analysis of MSC-Exos therapy for complex PFs. There are several limitations:(1) The absence of a control group, such as seton placement, surgical management or MSC application. So, we only conducted a single-arm meta-analysis to discuss the safety and efficacy of MSC-Exos for complex PFs, which limits the ability to draw definitive conclusions about efficacy and safety through inter-group comparisons. (2) The small sample size of included literature limits the precision of the estimates of effects of MSC-Exos therapy on complex PFs patients, most of them were retrospective studies. So, selection bias cannot be excluded, and extrapolation of the meta-analysis results was limited to some extent. (3) All patients were from Iran, and there may be ethnic differences. (4) The quality of the included studies was moderate. (5) The efficacy of exosomes derived from MSCs is contingent on multiple determinants. But subgroup analysis [e.g.: number of infusions, dosage of exosomes per infusion, the source of MSCs] was limited by the small size of the studies. Besides, the use of subgroup analyses could not account for all sources of heterogeneity. So, our study was limited by its multiple centers and heterogeneity in the study such as inclusion criteria, sample size, MSC-Exos origin, and injection frequency. However, we have carried out careful quality evaluation, sensitivity analysis, heterogeneity evaluation, and publication bias evaluation in this paper to make the data and conclusions as reliable as possible. In view of the current single-arm studies, confirmation of this conclusion will require more RCTs in the future. However, the existing evidence remains limited.

The potential of MSC-Exos for the treatment of complex PFs is exciting, but the difficulties are challenging. Despite their promise, MSC-Exos have limitations. Firstly, the isolation method of exosomes is required to be easily expandable to support large-scale manufacturing. MSCs are considered to be an attractive cell type for therapeutic exosome production. However, scalable methods to isolate and manufacture exosomes from MSCs are lacking. Low yields of exosomes impede the use of MSCs for exosome production. Haraszti RA et al study showed both 3D culture and tangential flow filtration improve the yield of exosomes to a cumulative extent of 140-fold [42]. However, this yield was only obtained in the laboratory and may not be sufficient for future standardized production for clinical use. Thus, there is an urgent need to develop production technologies that facilitate large-scale, stable generation of MSC-EXOs to fulfll therapeutic requirements. Secondly, future applications of MSC-Exos are most likely in combination with other drugs or systems. Now, there were some studies have demonstrated that MSC-Exos combined with drugs can further improve therapeutic outcomes in some diseases such as liver fibrosis [35].

However, the specific mechanisms by which combination therapy intervenes are unclear, which makes widespread clinical application difficult. Therefore, in future preclinical and clinical studies, the therapeutic mechanisms of MSC-Exos intervention in disease pathogenesis must be further elucidated. Thirdly, exosomes can be broadly classified into naturally occurring and engineered exosomes. Engineered exosomes offer distinct advantages and they can be modified by miRNA. Engineering exosomes with modified membrane proteins can further amplify therapeutic efficacy by enhancing targeting capabilities and cellular internalization. Although clinical trials on the use of MSC-Exos for complex PFs have been conducted, there is limited clinical research progress. Therefore, there remains a substantial shortfall in the clinical application of MSC-Exo, necessitating the conduct of more rigorous RCTs to bridge this gap.

5、Conclusions

Our meta-analysis demonstrates the efficacy and safety of MSC-Exos in patients with complex PFs, providing evidence for its future clinical application. Nevertheless, due to limited data, confirmation of this conclusion requires more RCTs. It is important to remind us that the use of MSC-Exos for complex PFs remains in the very early stages of treatment development. In the future, more clinical trials are needed to facilitate the widespread clinical adoption of MSC-Exos therapy.

References

1. Sainio P. Fistula-in-ano in a defined population. Incidence and epidemiological aspects. *Ann Chir Gynaecol*. 1984;73(4):219-224.
2. Eglinton TW, Barclay ML, Gearry RB, Frizelle FA. The spectrum of perianal Crohn's disease in a population-based cohort. *Dis Colon Rectum*. 2012;55(7):773-777.
3. American Gastroenterological Association Clinical Practice Committee. American Gastroenterological Association medical position statement: perianal Crohn's disease. *Gastroenterology*. 2003;125(5):1503-1507.
4. Sandborn WJ, Fazio VW, Feagan BG, Hanauer SB; American Gastroenterological Association Clinical Practice Committee. AGA technical review on perianal Crohn's disease. *Gastroenterology*. 2003;125(5):1508-1530.
5. Elahi KC, Klein G, Avci-Adali M, Sievert KD, MacNeil S, Aicher WK. Human Mesenchymal Stromal Cells from Different Sources Diverge in Their Expression of Cell Surface Proteins and Display Distinct Differentiation Patterns. *Stem Cells Int*. 2016;2016:5646384.
6. Melief SM, Geutskens SB, Fibbe WE, Roelofs H. Multipotent stromal cells skew monocytes towards an anti-inflammatory interleukin-10-producing phenotype by production of

interleukin-6. *Haematologica*. 2013;98(6):888-895.

7. Ocansey DKW, Pei B, Yan Y, et al. Improved therapeutics of modified mesenchymal stem cells: an update. *J Transl Med*. 2020;18(1):42. Published 2020 Jan 30.
8. Batrakova EV, Kim MS. Development and regulation of exosome-based therapy products. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2016;8(5):744-757.
9. Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. *Cells*. 2019;8(12):1605. Published 2019 Dec 11.
10. Wang X, Zhou Y, Ding K. Roles of exosomes in cancer chemotherapy resistance, progression, metastasis and immunity, and their clinical applications (Review). *Int J Oncol*. 2021;59(1):44.
11. Bazzoni R, Takam Kamga P, Tanasi I, Krampera M. Extracellular Vesicle-Dependent Communication Between Mesenchymal Stromal Cells and Immune Effector Cells. *Front Cell Dev Biol*. 2020;8:596079. Published 2020 Nov 6.
12. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. *Int J Mol Sci*. 2014;15(3):4142-4157. Published 2014 Mar 7.
13. Shen Z, Huang W, Liu J, Tian J, Wang S, Rui K. Effects of Mesenchymal Stem Cell-Derived Exosomes on Autoimmune Diseases. *Front Immunol*. 2021;12:749192. Published 2021 Sep 27.
14. Wu H, Fan H, Shou Z, et al. Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. *Int Immunopharmacol*. 2019;68:204-212.
15. Hu N, Cai Z, Jiang X, et al. Hypoxia-pretreated ADSC-derived exosome-embedded hydrogels promote angiogenesis and accelerate diabetic wound healing. *Acta Biomater*. 2023;157:175-186.
16. Liu H, Liang Z, Wang F, et al. Exosomes from mesenchymal stromal cells reduce murine colonic inflammation via a macrophage-dependent mechanism. *JCI Insight*. 2019;4(24):e131273. Published 2019 Dec 19.
17. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. Published 2021 Mar 29.
18. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*. 2016;355:i4919. Published 2016 Oct 12.
19. Nazari H, Alborzi F, Heirani-Tabasi A, et al. Evaluating the safety and efficacy of mesenchymal stem cell-derived exosomes for treatment of refractory perianal fistula in IBD patients: clinical trial phase I. *Gastroenterol Rep (Oxf)*. 2022;10:goac075. Published 2022 Dec 9.
20. Pak H, Hadizadeh A, Heirani-Tabasi A, et al. Safety and efficacy of injection of human placenta mesenchymal stem cells derived exosomes for treatment of complex perianal fistula in non-Crohn's cases: Clinical trial phase I. *J Gastroenterol Hepatol*.

2023;38(4):539-547.

21. Hadizadeh A, Akbari Asbagh R, Heirani-Tabasi A, et al. Localized Administration of Mesenchymal Stem Cell-Derived Exosomes for the Treatment of Refractory Perianal Fistula in Patients With Crohn's Disease: A Phase II Clinical Trial. *Dis Colon Rectum*. 2024;67(12):1564-1575.
22. Panés J, García-Olmo D, Van Assche G, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet*. 2016;388(10051):1281-1290.
23. Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant*. 2013;22(2):279-285.
24. Gnecchi M, Danieli P, Malpasso G, Ciuffreda MC. Paracrine Mechanisms of Mesenchymal Stem Cells in Tissue Repair. *Methods Mol Biol*. 2016;1416:123-146.
25. Gnecchi M, He H, Liang OD, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med*. 2005;11(4):367-368.
26. Arslan F, Lai RC, Smeets MB, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2013;10(3):301-312.
27. Kim DK, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ. Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. *Proc Natl Acad Sci U S A*. 2016;113(1):170-175.
28. Heldring N, Mäger I, Wood MJ, Le Blanc K, Andaloussi SE. Therapeutic Potential of Multipotent Mesenchymal Stromal Cells and Their Extracellular Vesicles. *Hum Gene Ther*. 2015;26(8):506-517.
29. Wei W, Ao Q, Wang X, et al. Mesenchymal Stem Cell-Derived Exosomes: A Promising Biological Tool in Nanomedicine. *Front Pharmacol*. 2021;11:590470. Published 2021 Jan 25.
30. Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transplant*. 2019;54(Suppl 2):789-792.
31. Lu Y, Huangfu S, Ma C, et al. Exosomes derived from umbilical cord mesenchymal stem cells promote healing of complex perianal fistulas in rats. *Stem Cell Res Ther*. 2024;15(1):414. Published 2024 Dec 29.
32. Larabi A, Barnich N, Nguyen HTT. Emerging Role of Exosomes in Diagnosis and Treatment of Infectious and Inflammatory Bowel Diseases. *Cells*. 2020;9(5):1111. Published 2020 Apr 30.
33. Wang Y, Zhang Y, Lu B, et al. hucMSC-Ex Alleviates IBD-Associated Intestinal Fibrosis by Inhibiting ERK Phosphorylation in Intestinal Fibroblasts. *Stem Cells Int*. 2023;2023:2828981.

Published 2023 Feb 17.

34. Tian S, Zhou X, Zhang M, et al. Mesenchymal stem cell-derived exosomes protect against liver fibrosis via delivering miR-148a to target KLF6/STAT3 pathway in macrophages. *Stem Cell Res Ther*. 2022;13(1):330. Published 2022 Jul 20.
35. Xu Y, Zhou X, Wang X, Jin Y, Zhou L, Ye J. Progress of mesenchymal stem cells (MSCs) & MSC-Exosomes combined with drugs intervention in liver fibrosis. *Biomed Pharmacother*. 2024;176:116848.
36. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *Int J Mol Sci*. 2017;18(9):1852. Published 2017 Aug 25.
37. Teixeira FG, Salgado AJ. Mesenchymal stem cells secretome: current trends and future challenges. Neural Regen Res. 2020;15(1):75-77.
38. Song Y, You Y, Xu X, et al. Adipose-Derived Mesenchymal Stem Cell-Derived Exosomes Biopotentiated Extracellular Matrix Hydrogels Accelerate Diabetic Wound Healing and Skin Regeneration. *Adv Sci (Weinh)*. 2023;10(30):e2304023.
39. Börger V, Bremer M, Ferrer-Tur R, et al. Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential as Novel Immunomodulatory Therapeutic Agents. *Int J Mol Sci*. 2017;18(7):1450. Published 2017 Jul 6.
40. Kang IS, Suh J, Lee MN, et al. Characterization of human cardiac mesenchymal stromal cells and their extracellular vesicles comparing with human bone marrow derived mesenchymal stem cells. *BMB Rep*. 2020;53(2):118-123.
41. Jung JW, Kwon M, Choi JC, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. *Yonsei Med J*. 2013;54(5):1293-1296.
42. Haraszti RA, Miller R, Stoppato M, et al. Exosomes Produced from 3D Cultures of MSCs by Tangential Flow Filtration Show Higher Yield and Improved Activity. *Mol Ther*. 2018;26(12):2838-2847.

Table 1. Characteristics of all included studies

Study	**Type of study**	**Cell type and source**	**Type of PFs**	**Mean age,years (mean ± SD)**	**Outcome assessment**	**the dosage of exosomes**	**Injection frequency**	**Study outcomes (at 6months)**	**AEs/SAEs**
Nazari H [19] (2022)	Phase I, clinical trial	human umbilical cord MSC	complex and refractory PF in CD	range 31–47years	physical examination+MRI	0.5×10^{10} particles/mL*5ml	Single	**complete healing:** 3/5 patients; 7/9 fistula tracts; **responded to treatment:** 4/5 patients; **no response:** 1/5 patient	NO
Pak H[20] (2023)	Phase I, clinical trial	human Placenta MSCs	complex PF in non-CD	range 35-56 years	physical examination+MRI	0.5×10^{10} particles/mL*5ml	repeated three times at weekly intervals	**complete healing:** 5/11 patients; 12/22 fistula tracts; **responded to treatment:** 10/11 patients; **no response:** 1/11 patient;	NO
Hadizadeh A [21] (2024)	Phase II, clinical trial	human umbilical cord MSC	complex and refractory PF in CD	34.52 ± 11.02 years	physical examination+MRI	0.5×10^{10} particles/mL*5ml	repeated three times at 2-month intervals	**complete healing:** 12/20 patients; 30/43 fistula tracts； **responded to treatment:** 16/20 patients; **no response:** 4/20 patients	NO

Table 2. ROBINS-I for risk of bias analysis

Bias domain	Nazari H (2022)	Pak H (2023)	Hadizadeh A (2024)
Bias due to confounding	Moderate	Moderate	Moderate
Bias in selection of participants into the study	Low	Low	Low
Bias in classification of interventions	Moderate	Moderate	Moderate
Bias due to deviations from intended interventions	Low	Low	Low
Bias due to missing data	Low	Low	Low
Bias in measurement of outcomes	Low	Low	Low
Bias in selection of the reported result	Low	Low	Low
Over all bias	Moderate	Moderate	Moderate

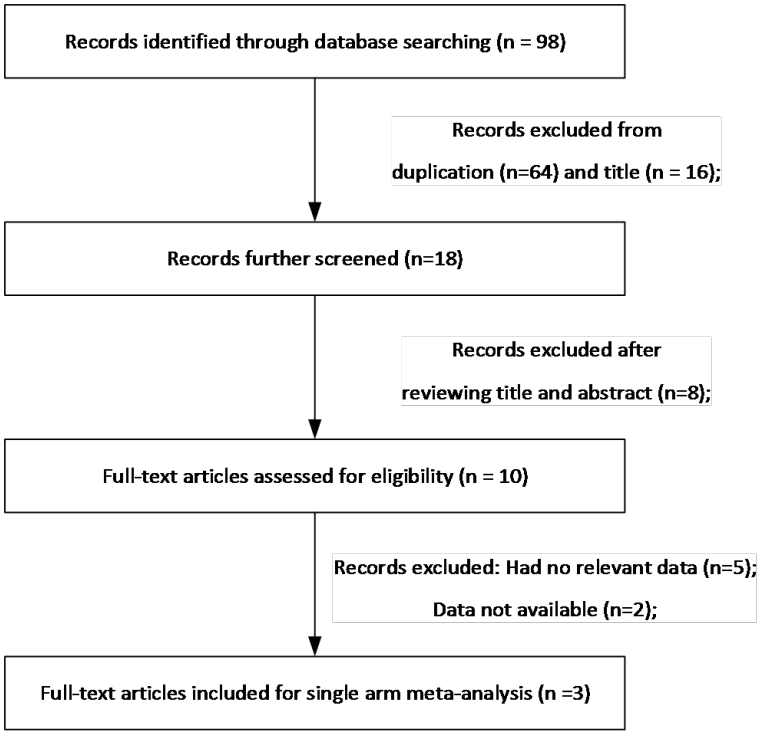

Fig. 1 Flow diagram of included and excluded studies in this meta-analysis.

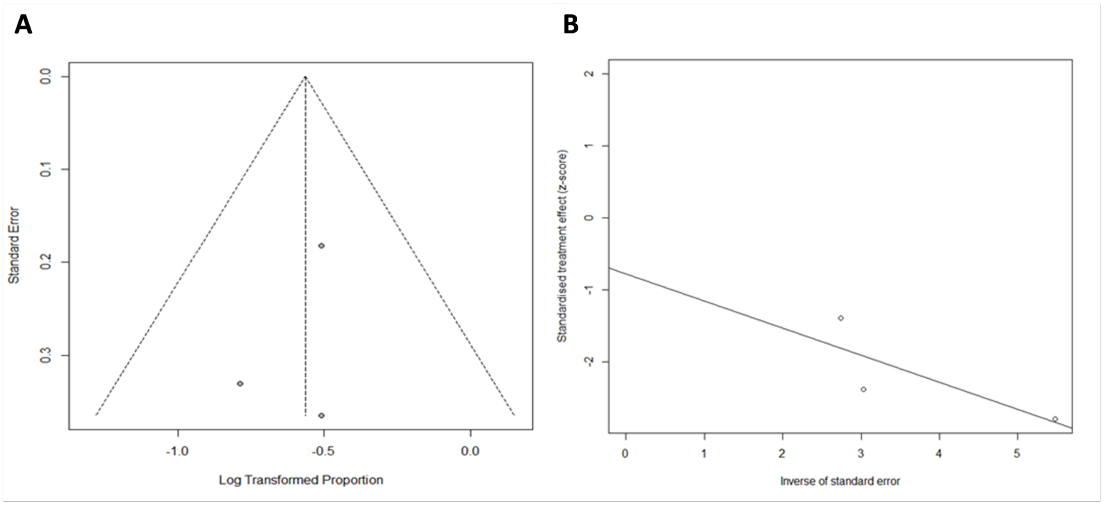

Fig 2A. Funnel plot shows no existence of publication bias.

Fig 2B. Egger's test was performed to detect publication bias for included studies.

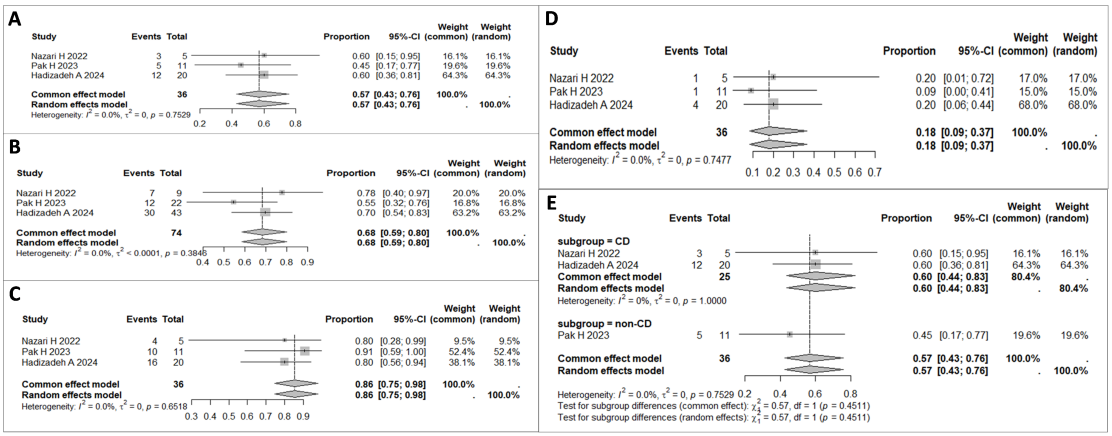

Fig.3A-B Forest plots about the pooled results of healing rate; Fig.3C Forest plots about the pooled results of clinical response; Fig.3D Forest plots about the pooled results of non-response; Fig.3E Forest plot showing changes in HR across different types of PFs (CD vs non-CD).