

Preliminary Characterization of Cervical Microflora in Pregnant Women with Cervical Incompetence

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Objective: The objective of this study was to investigate the impact of cervical microflora on cervical function and the efficacy of cervical cerclage in restoring the normal microecology of the reproductive tract, thereby improving pregnancy outcomes.

Methods: Based on defined inclusion and exclusion criteria, six pregnant women who underwent cervical cerclage for cervical incompetence at Shijiazhuang No. 4 hospital between January and May 2024 were enrolled. Cervical secretions were collected from each participant both prior to the procedure and one month afterward. High-throughput sequencing (Illumina MiSeq) was used to analyze the diversity and community structure of cervical microflora, enabling the assessment of changes associated with the cervical cerclage procedure.

Results: In this study, the composition of two groups of samples was analyzed at the “genus” level. *Lactobacillus* spp. predominated both pre- and post-operative samples. In the postoperative period, there was a decrease in the relative abundance of *Gemella*, *Rhodococcus*, and *Anaerococcus*, while an increase in the relative abundance of *Bifidobacterium* was noted. When the composition of the flora of two groups of samples was analyzed at the “phylum” level, the top four dominant bacteria with the highest abundance were *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*, with *Firmicutes* being the most abundant, and there was no significant difference between the two groups. However, there was a gradual increase in the abundance of *Actinobacteria* and a decrease in *Proteobacteria* and *Bacteroides* over time.

Conclusion: The results of this study are useful in exploring the effect of cervical flora on outcomes in cervical cerclage, potentially contributing to accelerating the restoration of normal vaginal microecology and improving pregnancy outcomes.

Keywords: cerclage, cervical incompetence, flora variability, Illumina MiSeq, microecology

Introduction

The prevalence of cervical insufficiency in the general obstetric population is about 0.5%. However, this prevalence increases to about 8% in individuals with a previous history of pregnancy loss due to abortion.^{1,2} Approximately half of preterm deliveries are associated with microbiological factors, specifically pathological changes in the microflora of the reproductive tract.^{3,4} Pregnant women who have preterm deliveries exhibit a greater abundance of vaginal flora and a higher Shannon's index in late pregnancy compared to non-preterm pregnant women.^{5,6}

Over 50% of the preterm deliveries worldwide occur in Asia, with cervical incompetence (CIC) being an important cause of miscarriages and preterm deliveries in the second trimester.⁷ The underlying mechanism of its occurrence may involve disruptions in the genital tract flora, which allows the secretion of pro-inflammatory factors and causes collagen lysis of the cervical mesenchyme, leading to cervical insufficiency and a likely increase in the risk of midterm miscarriage and preterm delivery. Although bacteria and yeast are considered part of the normal flora of both the vagina and the cervix, it remains great significance to characterize the microflora in pregnant women with cervical incompetence. In this article, the impact of cervical microflora on cervical function and cervical cerclage has been explored with the aim of restoring the normal microecology of the reproductive tract and enhancing pregnancy outcomes.

Data and Methods

General Data

The study sample consisted of six pregnant women who underwent cervical cerclage for cervical incompetence at the Shijiazhuang No. 4 hospital between January 2024 and May 2024. Cervical secretions were collected before the procedure and one month post-surgery.

The inclusion criteria were as follows: (1) patients who fulfilled the diagnostic criteria outlined in the *Clinical Practice Guidelines for Cervical Incompetence and Cervical Cerclage*⁷ were included in the study group, while the control group comprised healthy pregnant women who had full term deliveries; (2) women who had a single first-trimester pregnancy; (3) women who had conceived naturally; and (4) those who were ≥ 18 years old.

The exclusion criteria were as follows: (1) co-existing hyperthyroidism, cervical cancer, or other neoplastic diseases; (2) co-existing immunodeficiency, infectious diseases, or hematologic diseases; (3) presence of peripheral neurological diseases, psychiatric disorders, or cervicitis; (4) important organ dysfunction and communication disorders; (5) malformation of the reproductive organs; (6) abnormal development or cessation of fetal development; (7) patients who were administered antibiotics, antivirals, antifungals, etc. in the previous month; (8) those with pregnancy complications such as hyperosmolar coma, ketoacidosis, gestational diabetes mellitus, and gestational hypertension.

The study was approved by the Medical Ethics Committee of the hospital (Approval number: 2023–028), and all patients and their families signed an informed consent form.

Specimen Collection, Sequencing, and Analysis

Preoperative and postoperative cervical secretion samples were collected from pregnant women with cervical incompetence using sterile cotton swabs, which were placed in tubes that contain preservation solution. The specimens were immediately stored in a refrigerator at -4°C and later transferred to a -80°C freezer for storage until DNA extraction. Genomic DNA was extracted and detected using 1% agarose gel electrophoresis.

High-throughput 16S rRNA gene sequencing method was used to detect and compare the microorganisms in preoperative and postoperative cervical mucus samples. 16S rRNA gene sequencing was performed using 16S rRNA gene PCR primers: Forward primer, 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGNGGC WGC AG-3'; reverse primer, 5'-GTC TCG TGG GCT CGG GA TGT GTA TAAGAG ACA GGA CTA CHV GGG TAT CTAATCC-3'. Trizol was used to extract DNA from cervical mucus. Illumina and other platforms were used for high-throughput library sequencing to obtain a large number of 16S rRNA gene sequence data. Quantitative Insights for Microbial Ecology, Uparse, and RDP were used for sequence assembly and classification analysis. Classifiers perform OTU clustering and sequence classification annotation. To measure beta diversity, the Bray-Curtis distance was used to identify differences in biological composition. Then, principal component analysis was performed using beta diversity results.

Results and Analysis

The samples of six pregnant women who were scheduled to undergo cervical cerclage were divided into two groups: the preoperative group (Pre) and the postoperative group (Post). Cervical secretions were collected before the operation and one month after the procedure. The samples were sequenced following DNA extraction and library construction. The total number of sequences was 356,598, with a mean value of 59,433, showing no significant difference in the distribution of sequences. The sequences obtained were clustered into OTUs with 97% similarity. This clustering demonstrated that the pre-surgery cervical flora richness in the group of pregnant women was higher than that in the post-operative group of pregnant women, as illustrated in [Figure 1](#).

Alpha Diversity Analysis

In this study, the Shannon Index was used to assess the alpha diversity of the flora composition. The Shannon Index of the flora in the preoperative group (Shannon Index = 1.1439 ± 1.3991) was significantly higher than that of the postoperative group (Shannon Index = 0.64441 ± 0.047711), and this difference was statistically significant, as illustrated in [Figure 2](#).

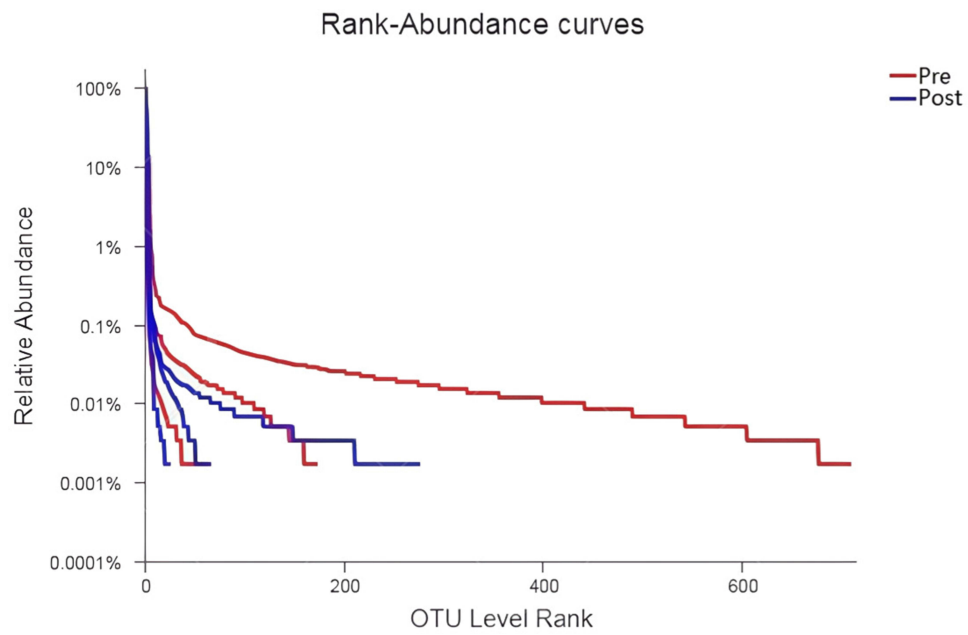


Figure 1 Cervical flora richness in pregnant women group before surgery.
Abbreviation: OTU, operational taxonomic unit.

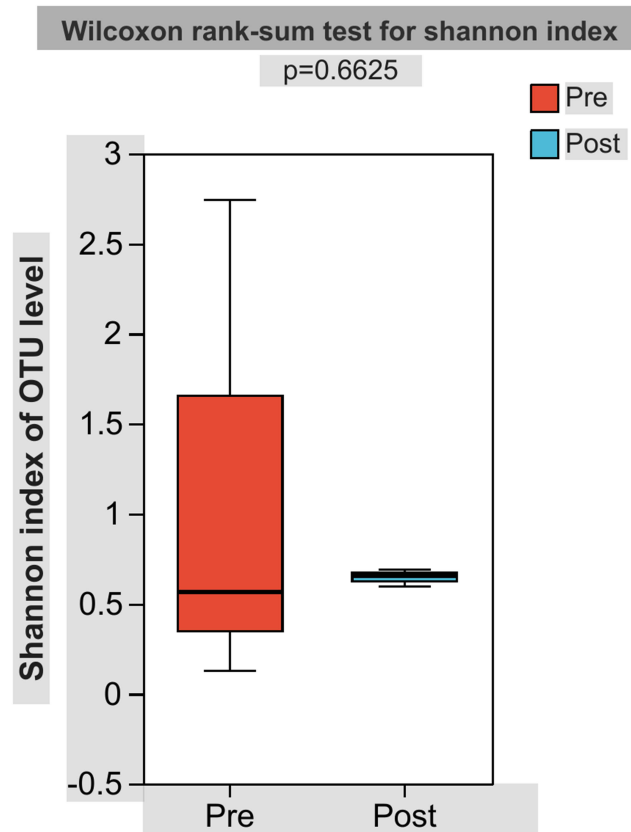


Figure 2 Comparison of Shannon index before and after surgery.
Abbreviation: OTU, operational taxonomic unit.

Beta-Diversity Analysis

Based on the weighted UniFrac value, Principal Coordinates Analysis (PCoA) was performed, and the results are presented in Figure 3 below. The analysis revealed a distinction between the bacterial flora of the preoperative and postoperative groups, indicating significant changes in the flora composition following the cervical cerclage procedure.

Analysis of Flora Composition

In this experiment, we identified a total of 846 different OUTs, including one domain, one kingdom, 20 different phyla, 33 different classes, 75 different orders, 120 different families, 251 different genera, and 403 different species.

At the genus level of classification, the five most prevalent microorganisms were *Lactobacillus*, *Bifidobacterium*, *Gemella*, *Rhodococcus*, and *Anaerococcus*. At the phylum level of classification, as shown in Figure 4, the five most common microorganisms were *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroides*, and Unclassified Bacteria.

As shown in Figure 5a, there were 240 unique strains in the preoperative group and 10 unique strains in the postoperative group, totaling 153 strains. As seen in Figure 5b and c, the most abundant species in the preoperative group were hydrogen-producing *Anaerococcus*, accounting for 80.05%. In contrast, the most abundant species in the postoperative group was *Bifidobacterium*, accounting for 99.13%. Interestingly, *Lactobacillus* was the most abundant species in both groups, representing 46.95%.

The analysis of the composition of two groups of samples at the “genus” level revealed that *Lactobacillus spp.* predominated both before and after surgery. In the postoperative period, there was a decrease in the relative abundance of *Gemella*, *Rhodococcus*, and *Anaerococcus* and an increase in the relative abundance of *Bifidobacterium*.

When the composition of the flora of two groups of samples was analyzed at the “phylum” level (Figure 6a and b), the top four dominant bacteria with the highest abundance were *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and

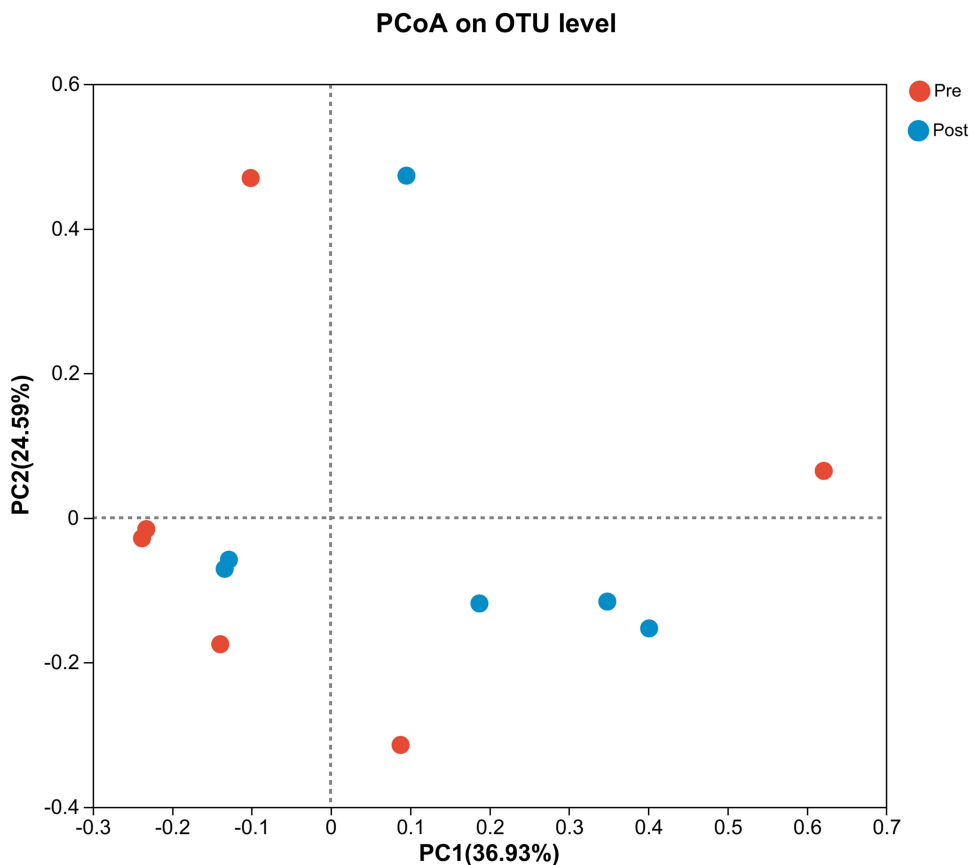


Figure 3 Pre- and post-operative (PCoA) analysis.

Abbreviations: OTU, operational taxonomic unit; PCoA, Principal Coordinates Analysis.

Community analysis pieplot on Phylum level

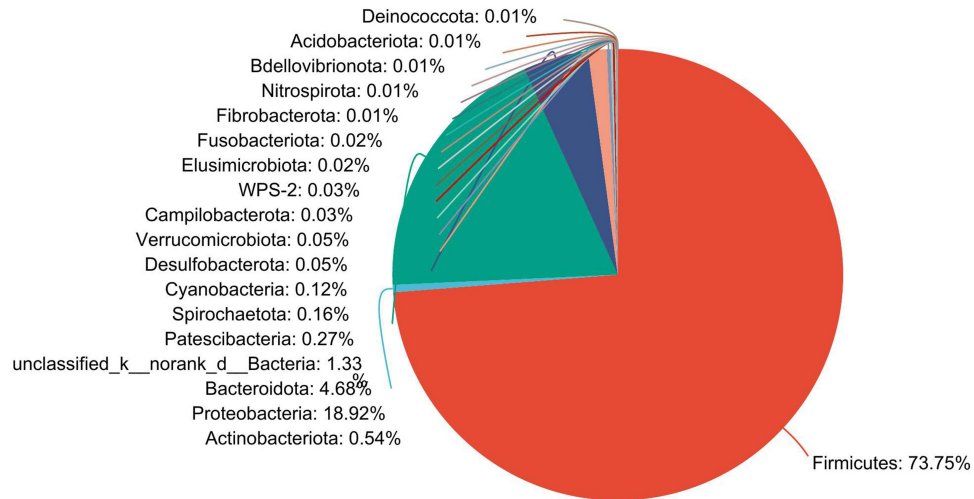


Figure 4 Classification of portal levels.

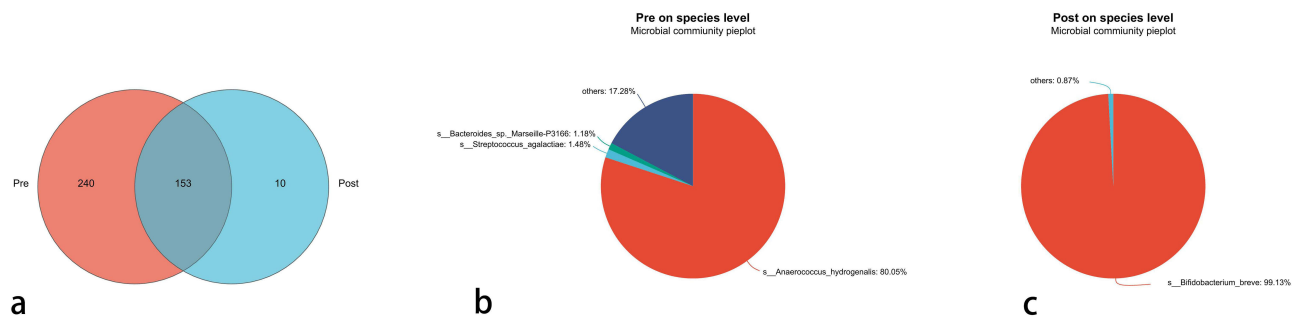


Figure 5 (a) Pre- and postoperative flora characteristics; (b) Percentage of flora in the preoperative group; (c) Percentage of flora in the postoperative group.

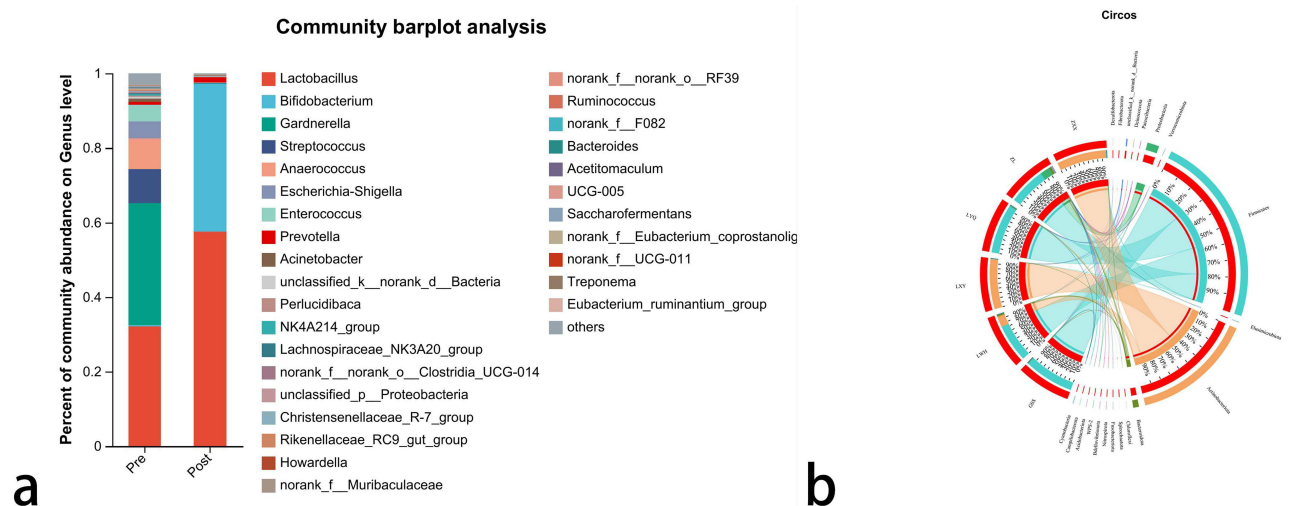


Figure 6 (a) Comparison of bacterial flora richness before and after surgery; (b) Bacterial flora richness circos plot.

Bacteroidetes, with *Firmicutes* having the highest abundance, and there was no significant difference between the two groups. Over time, the abundance of *Actinobacteria* gradually increased, while the abundance of *Proteobacteria* and *Bacteroides* decreased.

Discussion

Of late, the study of the correlation between reproductive tract flora and pregnancy outcomes has gradually garnered increasing academic interest. Abnormal genital tract microflora has been shown to adversely affect pregnancy outcomes, especially in pregnant women who have undergone cervical cerclage. Maintaining a favorable vaginal environment appears to be critical. Abnormalities in the anatomy or function of the cervix, such as cervical insufficiency, manifest as clinical conditions like cervical shortening, dilatation, and effacement during pregnancy. These abnormalities can lead to the bulging of the amniotic sac, consequently increasing the incidence of perinatal complications.⁸

During a normal pregnancy, the cervix plays a crucial role in protecting the uterus and fetus from infections originating upstream and acts as a functional physical barrier against bacteria and pathogens.⁹ However, in cases of cervical incompetence, microorganisms can invade through the compromised cervical barrier, resulting in amniotic cavity infection, inflammatory activation, and preterm labor.¹⁰ Cervical incompetence is a significant cause of mid-pregnancy miscarriage and preterm labor. It is estimated that up to 1% of pregnant women have cervical incompetence, and this condition occurs in 8% of women who experience recurrent miscarriages.¹¹ The actual incidence of cervical incompetence may be underestimated due to the diagnostic challenges associated with it.

Currently, it has been found in some international studies that pregnant women with cervical incompetence may experience changes in their vaginal microflora after undergoing cerclage.¹² These findings have indicated that alterations in microflora before and after the procedure could potentially be linked to improved pregnancy outcomes. However, whether altering the genital tract microflora would improve the therapeutic efficacy of cerclage and thus further enhance pregnancy outcomes was not directly validated in these investigations.

In our study, the flora composition and abundance of pregnant women with cervical insufficiency before and after surgery were analyzed. We found significant differences in the composition and abundance of flora pre- and post-surgery. Notably, there was an increase in the abundance of certain beneficial bacterial species post-surgery, while that of harmful species decreased. Since vaginal disinfection was performed during surgery, the microbiota was expected to change. Additionally, individual variations may lead to differences in the vaginal microbial environment.

These changes may help explain why some pregnant women who underwent cerclage experienced improved pregnancy outcomes after the surgery.

A limitation of the current study is the small sample size, which may not fully represent the broader population of all pregnant women with cervical incompetence. Therefore, expanding the sample size further is necessary to comprehensively examine the relationship between reproductive tract microflora characteristics and preterm delivery in pregnant women with cervical incompetence. How changes in specific microbial communities affect pregnancy outcomes, particularly the incidence of preterm labor and other perinatal complications, should be focused upon in future studies. In addition, investigations can explore whether methods to improve the microflora, such as probiotic therapy, could positively impact pregnancy outcomes in pregnant women with cervical incompetence. Such studies can provide significant insights for the development of new clinical treatment strategies to help reduce preterm labor and other complications, ultimately improving pregnancy success. Moreover, there is no control group included in this study. Future studies comparing women with cervical incompetence and those without are needed. Lastly, this study is currently in its preliminary testing phase due to the extended time required for comprehensive analysis. Specimen collection is ongoing, and only partial results are presented here.

Conclusion

In conclusion, our study shows that *Lactobacillus spp.* predominated both pre- and post-operative samples. In the postoperative period, there was a decrease in *Gemella*, *Rhodococcus*, and *Anaerococcus*, while an increase in *Bifidobacterium*. The top four dominant bacteria with the highest abundance were *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*, with *Firmicutes* being the most abundant. However, there was a gradual increase in the abundance of *Actinobacteria* and a decrease in *Proteobacteria* and *Bacteroides*. These findings provide a valuable reference for further exploration into and addressing the mechanisms of preterm labor. By continuously enhancing their understanding of the microflora, scientists and physicians can develop more effective interventions to reduce pregnancy-related risks and improve the overall health of pregnant women and newborns.

Data Sharing Statement

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Shijiazhuang Fourth Hospital (Approval number: 2023–028). A written informed consent was obtained from all participants.

Consent for Publication

Consent for publication was obtained from every individual whose data are included in this manuscript.

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Disclosure

None of the authors have any financial disclosure or conflict of interest.

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