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Original Article

Prevalence of vaginal colonizing microbiota among pregnant mothers visiting Nakaseke Hospital. A cross-sectional study.

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Abstract

Background:

This study aimed to determine the prevalence and diversity of vaginal colonizing microbiota among pregnant women attending Nakaseke Hospital in Uganda.

Methodology:

A cross-sectional study design was adopted, involving 144 pregnant women aged 18- 45 years attending antenatal care at Nakaseke Hospital between July and August 2025. Participants were selected through convenience sampling, with inclusion criteria ensuring they were at least 12 weeks pregnant and not on antibiotics recently. Vaginal swabs were collected aseptically and cultured using standard microbiological techniques, including Gram staining, biochemical tests, and culture media for bacteria and fungi. Data analysis was performed using descriptive statistics, with results presented in tables and figures.

Results:

The findings revealed that over half (55.6%) of the pregnant women harbored detectable microbiota, with *Candida albicans* being the most prevalent organism (15.2%), followed by *Gardnerella vaginalis*, *Escherichia coli*, and *Streptococcus agalactiae*. Microbial diversity varied notably with maternal age and parity; younger women (18–24 years) predominantly carried L*actobacillus spp*., which are protective, whereas women aged 35 and above exhibited higher prevalence of *Candida albicans* and absence of *Lactobacillus*, indicating increased risk for fungal infections. Parity also influenced microbiota composition, with multiparous women showing greater microbial diversity and higher colonization by pathogenic organisms compared to primiparous women.

Conclusion:

Findings underscore significant variations in vaginal microbiota among pregnant women in Nakaseke, influenced by age and parity, which may impact pregnancy outcomes. The high prevalence of potentially pathogenic organisms highlights the need for routine screening and targeted interventions to promote vaginal health.

Recommendations:

Implement microbiota monitoring during pregnancy, promote probiotic use to support beneficial Lactobacillus colonization, and conduct longitudinal studies to better understand microbiota dynamics and their health implications. This localized data provides a foundation for improving maternal healthcare strategies in rural Uganda and similar settings.

Keywords: *Prevalence, Vaginal colonizing microbiota, Pregnant mothers, Nakaseke Hospital.*
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Background

Globally, research has shown that a healthy vaginal microbiome is predominantly composed of Lactobacillus species, which can account for 70-90% of the vaginal flora in healthy women (Auriemma et al., 2021). However, studies have found that 20-30% of women in various populations experience dysbiosis, characterized by a decrease in Lactobacillus and an overrepresentation of harmful bacteria. For instance, an analysis of pregnant women in different countries has shown that 15% to 40% may have bacterial vaginosis (Romero et al., 2023; Shabayek, Abdellah, Salah, Ramadan, & Fahmy, 2022)

In Africa, the prevalence of Lactobacillus species in the vaginal microbiome is notably lower compared to other regions. Auriemma (2021) suggests that up to 50% of women in certain regions face vaginal health issues, resulting from socio-economic factors, healthcare access, and cultural practices that influence hygiene and reproductive health and notes that microbiota diversity tends to be higher among African women, with a greater representation of anaerobic bacteria, which can lead to an increased risk of BV and associated complications during pregnancy(Auriemma et al., 2021). This diversity is compounded by factors such as diet, healthcare access, and

environmental conditions that influence microbial colonization. A recent report revealed that about 40% of pregnant women in sub-Saharan Africa are affected by bacterial vaginosis, which poses serious risks for maternal and neonatal health outcomes (Baud et al., 2023).

Sub-Saharan Africa mirrors the broader African trends, with studies indicating a significant prevalence of diverse vaginal microbiota compositions. In a recent study, Baud (2023) emphasized that in Sub-Saharan Africa, socio-economic factors and access to healthcare directly impact the vaginal microbiome's health. Women in this region often experience higher rates of STIs and reproductive health issues, which are exacerbated by the presence of dysbiotic microbiomes (Baud et al., 2023).

Research indicates that 25% to 35% of women of reproductive age in sub-Saharan Africa present with microbial vaginosis, a condition linked to preterm birth and adverse pregnancy outcomes (Baud et al., 2023; Hashiramoto et al., 2023). Sub-Saharan Africa has seen limited access to healthcare, further intensifying the reproductive health challenges women face.

A study conducted by Hashiramoto (2023) reveals that these differences may lead to adverse outcomes such as preterm birth, maternal morbidity, and neonatal infections, highlighting the critical need for interventions that promote vaginal health among pregnant women in these regions (Hashiramoto et al., 2023).

In Uganda specifically, studies suggest that factors such as high rates of adolescent pregnancies, low access to quality prenatal care, and cultural variations in health-seeking behaviors contribute to microbial imbalances. A 2021 study indicated that nearly 25% of pregnant women in urban areas of Uganda exhibited symptoms associated with vaginal infections (Juliana & Peters, 2021). Furthermore, Mulinganya (2021) reported that only 15% of women in rural areas engage meaningfully with maternal healthcare services, potentially exacerbating the prevalence of untreated infections (Mulinganya et al., 2021).

Within this broader context, Nakaseke District presents a unique case for examining the vaginal microbiome composition of pregnant women. This area, characterized by its rural setting and socioeconomic challenges, has limited healthcare infrastructure and awareness regarding maternal health. Preliminary data suggest that approximately 30% of pregnant women in Nakaseke may experience alterations in their vaginal microbiome, with factors such as nutrition and sanitation contributing to the microbiome's diversity (Bwanga et al., 2023).

Significantly, recent analyses indicated that approximately 40% of pregnant women in Nakaseke District reported symptoms of infections during pregnancy, with bacterial vaginosis being prevalent among 15% of those cases (Ainomugisha et al., 2023; Fardi, Nur, & Hestiani, 2024). This highlights an urgent need for localized research to accurately depict the vaginal microbiome composition among pregnant women in Nakaseke. This study aimed to investigate the vaginal microbiome composition in pregnant women in Nakaseke District.

Methodology

Study Area

The study was conducted at Nakaseke Hospital, a rural public hospital in the Central Region of Uganda, built in the 1960s by the administration of Prime Minister Milton Obote. It serves Nakaseke District together with some parts of the neighboring districts of Luweero, Wakiso, Nakasongola and Mityana. The hospital is located in the town of Nakaseke, in Nakaseke District, in the part of the country known as the Luweero Triangle. Its location is approximately 65 kilometres (40 mi), by road, northwest of Mulago National Referral Hospital. This is approximately 35 kilometres (22 mi) by road, northwest of Bombo Military Hospital, the nearest bigger hospital. The coordinates of Nakaseke General Hospital are: 0°43'06.0"N, 32°23'56.0"E (Latitude:0.718344; Longitude:32.398893).

On average, the centre receives about 350 patients per day. It offers different types of services such as laboratory, pharmacy, surgical, counseling, and cervical cancer screening.

Study Design

A cross-sectional design was utilized to collect data from pregnant women attending the antenatal care clinic over a three-month period. This is because the study investigated the prevalence of Vaginal colonizing Bacteria by age in a specified period of time.

Study Population

The population comprised approximately 200 pregnant women aged 18-45 who met the inclusion criteria.

Inclusion Criteria

Women aged 18- 45 years.
Pregnant women at least 12 weeks into gestation.
Participants must provide informed consent.

Exclusion Criteria

Women with known vaginal infections at the time of sampling.
Women who have taken antibiotics within the last 30 days.

Sampling Technique

Convenience sampling was adopted to recruit participants over the study period, ensuring representation from diverse demographics.

Sample Size Estimation

Sample size = 4pq / L2 (Martin, Chen, Koehren, & Pereira, 1994)
Where:

n=sample size required
p=estimated prevalence of Vaginal bacterial colonization among pregnant women in Nakaseke District. q=1-p
L=desired error (required precision)
Since there is no recent publication about the prevalence of Vaginal bacterial colonization among pregnant women in Nakaseke District Hospital, a prevalence of 10% was used to calculate the sample size with a desired error of 5% (0.05).
$n=4x0.1(1-0.1) / 0.05^2$
n=144.
Thus, a minimum sample size of 144 was needed.

Sampling Technique
The study employed use of a convenience sampling technique to select the samples. It's preferred to other techniques because it ensures that each respondent has an equal chance of being selected, hence eliminating bias.

Sampling procedure
Patients who met the study criteria and were available for participation were selected. Since the target population is specific and identifiable, only eligible individuals were recruited.

Data Collection Tools
Data were gathered through a structured request form and microbiological analysis of vaginal swab samples.
Data were collected using the following tools:
Laboratory Investigation Request Form: This form was used to gather essential demographic information and clinical history.

Data Entry Sheet: A data entry sheet was implemented for systematic recording of all obtained data during the study, allowing for effective organization and analysis post-collection. These tools ensured comprehensive data gathering for the study's primary objectives, effectively addressing the research questions concerning the prevalence and composition of vaginal microbiota among pregnant mothers attending Nakaseke Hospital.

Data Collection Procedures
An introductory letter was obtained from the Mildmay Institute of Health Sciences and presented to the Medical Director of Nakaseke Hospital to facilitate access to respondents for data collection. Data were collected using a structured questionnaire administered to participants through face-to-face interviews. Participants were selected using convenience sampling from the different clinics. Data were collected, analyzed, interpreted, bound, and represented as a report.

Laboratory Procedures
Sample Collection and Processing
Sample Collection: A healthcare provider collects a vaginal swab using a sterile technique to minimize contamination.
Transport to Laboratory: The swab is placed in a sterile transport medium and sent to the laboratory for analysis.

Inoculation
Aseptic Technique: Laboratory personnel use aseptic techniques to prevent contamination.
Media Selection: Appropriate culture media are chosen based on suspected pathogens:
Blood Agar: For a wide range of aerobic and anaerobic bacteria.
Chocolate Agar: For fastidious organisms like Haemophilus and Neisseria species.
MacConkey Agar: For Gram-negative bacteria, differentiating lactose fermenters from non-fermenters.
Candida-selective Media: For suspected fungal infections.
Inoculation Process: The swab is streaked across the agar surface to promote the growth of individual colonies.

Incubation
Conditions: Plates are incubated at 35–37°C for 24 to 48 hours; some cultures may require specific conditions (e.g., anaerobic environments).
Observation of Growth: After incubation, plates are examined for colony growth.

Identification of Microorganisms
Colony Morphology: Colony size, color, and morphology provide initial clues about the type of microorganisms present.
Gram Staining: A Gram stain is performed to distinguish between Gram-positive and Gram-negative organisms. This helps narrow down potential pathogens.
Biochemical Tests: Identification involves biochemical tests (e.g., catalase, oxidase, urease tests) that assess metabolic characteristics unique to various species.

Sensitivity Testing
Antimicrobial Susceptibility Testing: If pathogenic bacteria are identified, susceptibility to antibiotics is tested using methods such as disk diffusion or broth microdilution.

Interpretation of Results
Analysis of Culture Results
Positive vs. Negative Culture: A positive culture indicates the presence of microorganisms, while a negative culture suggests no significant growth of the pathogens.
Quantification: Results may indicate the quantity of organisms (e.g., heavy, moderate, or light growth). Heavy growth could imply an infection, while light growth may be normal flora or contamination.

Identification Report: The results specified the microorganisms identified, their characteristics (e.g., whether they are pathogenic), and any relevant biochemical test outcomes.

Clinical Relevance

Pathogenic Species: Isolated organisms, such as *Candida albicans*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, and *Streptococcus agalactiae*, are clinically significant and may require treatment.
Normal Flora: Commensal organisms like Lactobacillus species may be present but are not pathogenic; they help maintain a healthy vaginal microbiota.

Reference ranges and Interpretation

Normal Flora: In a healthy vagina, common findings may include:
High levels of Lactobacillus species.
Absence or low levels of pathogenic organisms.
Abnormal Findings: The presence of high quantities of certain bacteria or other organisms could indicate:
Bacterial Vaginosis: Associated with high levels of *Gardnerella vaginalis* and a decrease in Lactobacillus.
Vulvovaginal Candidiasis: Characterized by the presence of Candida species.
Trichomoniasis: Presence of *Trichomonas vaginalis*.

Piloting Study

The questionnaire was pretested at Mildmay Institute of Health Sciences before data collection at the study site. This helped identify potential challenges and refine the questionnaire to ensure clarity and accuracy. Based on the findings, necessary adjustments such as revising questions, improving training, or streamlining procedures were made to ensure the main study runs smoothly and yields reliable results.

Quality Control

Quality Control for Media and Reagents
Sterility Checks: All culture media were checked for sterility before use. Control plates were incubated separately to ensure no contamination occurred during media preparation.
Use of Control Strains: Known control strains were used alongside patient samples to validate the accuracy and effectiveness of biochemical tests and culture techniques.
Personnel Training
Training Programs: Laboratory personnel had undergone training and competency assessments to ensure proper handling and processing of samples.
Equipment Calibration
Regular Calibration: Incubators, autoclaves, and other equipment were regularly calibrated and maintained to ensure optimal performance.
Documentation
Records Maintenance: Accurate records were maintained for all quality control tests performed, including test results, reagents used, and any deviations from protocols.

Data Analysis and Processing

Data was analyzed using a computer program known as Microsoft Excel and presented using frequency tables, pie charts, and bar graphs.

Ethical considerations

Before commencing the study, a letter of introduction from Mildmay Institute of Health Sciences and a copy of the research report were presented to Mengo Hospital administration for approval.
Participants were informed about the study's objectives, and their informed consent was obtained before participation. Confidentiality and anonymity were strictly maintained.

Results

Prevalence of vaginal microbiota among pregnant mothers attending Nakaseke Hospital from July to August 2025

Organism	Number of Participants	Percentage (%)
Candida albicans	22	15.2
Gardnerella vaginalis	16	11.1
Lactobacillus spp.	14	9.7
Escherichia coli	15	9.6
Streptococcus agalactiae	14	9.7
Total Prevalence	**80**	**55.6**
No organism isolated	64	44.4

Table 1: Showing the prevalence of vaginal microbiota among pregnant mothers attending Nakaseke Hospital from July to August 2025.

Overall, more than half of the participants harbored detectable organisms, indicating diverse vaginal microbiota, with a substantial proportion of 55.6% showing colonization by organisms associated with infections or dysbiosis. This data illustrates that a diverse range of microbial organisms colonize the vaginal environment of pregnant women attending Nakaseke Hospital, with *Candida albicans* (15.2%) being the most prevalent among detected organisms.

Microbial diversity present in the vaginal samples among pregnant mothers attending Nakaseke Hospital from July to August 2025.

Organism	Number of Participants	Percentage (%)
Candida albicans	22	15.2
Gardnerella vaginalis	16	11.1
Lactobacillus spp.	14	9.7
Escherichia coli	15	9.6
Streptococcus agalactiae	14	9.7
No organism isolated	64	44.4

Table 2: Showing microbial diversity present in the vaginal samples among pregnant mothers attending Nakaseke Hospital from July to Aug 2025.

A total of 144 participants were included in the analysis. Among the detected organisms, Candida albicans was the most prevalent, identified in 15.2% of participants. *Gardnerella vaginalis* and *Streptococcus agalactiae* were both observed in approximately 11% and 9.7% of samples, indicating their potential role in bacterial vaginosis and neonatal infections, respectively. *Escherichia coli* and *Lactobacillus spp.* were also present in 9.6% and 9.7% of samples, reflecting common bacterial colonizers. Overall, these findings underscore the diversity of vaginal microbial flora in pregnant women and highlight the importance of microbial monitoring for maternal and neonatal health.

Distribution of Vaginal Microbiota by Maternal Age Group and vaginal microbiota composition among pregnant women at Nakaseke Hospital.

Age Group	No. of Participants	*Lactobacillus spp.*	*Gardnerella vaginalis*	*Candida albicans*	*Escherichia coli*	*Streptococcus agalactiae*
18-24	10	5 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
25-29	71	7 (9.8%)	2 (2.8%)	6 (8.4%)	6 (8.4%)	1 (1.4%)
30-34	53	2 (3.7%)	12 (22.6%)	8 (15%)	8 (15%)	13 (24.5%)
35+	10	0 (0%)	2 (20%)	8 (80%)	0 (0%)	0 (0%)

Table 3: Showing Distribution of Vaginal Microbiota by Maternal Age Group and vaginal microbiota composition among pregnant women at Nakaseke Hospital.

Table 3 presents the distribution of vaginal microbiota among pregnant women at Nakaseke Hospital, stratified by maternal age groups. The data highlight the prevalence of various microbial species within the vaginal environment across different age ranges.

In the 18-24 age group, half of the participants (50%) exhibited a microbiota dominated by Lactobacillus spp., which is generally considered beneficial for vaginal health.

The 25-29 age group showed a diverse microbiota profile, with Lactobacillus spp. present in approximately 9.8% of women. Notably, Gardnerella vaginalis and Candida albicans were detected in 2.8% and 8.4%, respectively, indicating some dysbiosis or potential infections. Escherichia coli and Streptococcus agalactiae were found in 8.4% and 1.4%, respectively.

Women aged 30-34 exhibited a higher prevalence of Gardnerella vaginalis (22.6%) and Streptococcus agalactiae (24.5%), suggesting an increased risk of bacterial vaginosis and Group B Streptococcus colonization in this group. Candida albicans was also present in 15%, and Lactobacillus spp. were detected in a small percentage (3.7%).

In the 35+ age group, the majority of microbiota profiles were characterized by Candida albicans (80%), indicating a significant presence of fungal colonization. Gardnerella vaginalis was present in 20%, while Lactobacillus spp. were absent, which could suggest age-related shifts in vaginal flora potentially impacting maternal health.

Distribution of Vaginal Microbiota by Maternal Parity and vaginal microbiota composition among pregnant women at Nakaseke Hospital from Jul to Aug 2025.

Parity	*Lactobacillus spp.(%)*	*Candida albicans(%)*	*Gardnerella vaginalis(%)*	*Escherichia coli(%)*	*Streptococcus agalactiae (%)*
0	2(7.1)	0	0	1(3.7)	0
1	8 (22.2)	4 (11.1)	0	1 (2.7)	0
2	4 (10.8)	3 (8.1)	8 (21.6)	9 (24.3)	1(2.7)
3+	0	15 (34.8)	8 (18.6)	3(6.9)	13 (30.2)

Table 4: Showing Distribution of Vaginal Microbiota by Maternal Parity and vaginal microbiota composition among pregnant women at Nakaseke Hospital from July to August 2025.

Primiparous women showed low prevalence of *Lactobacillus spp*., with the majority having no detectable vaginal microbiota growth, suggesting a less protective microbiota profile.

Women with one previous birth exhibited increased *Lactobacillus spp*. presence and some *Candida albicans*, indicating a shift toward a more balanced microbiota, but still considerable no-growth cases.

Multiparous women had higher diversity with a notable presence of *Gardnerella vaginalis, Escherichia coli*, and other bacteria, reflecting increased microbiota variability with parity.

Women with three or more prior births showed a significant increase in *Candida albicans and Streptococcus agalactiae*, indicating a possible higher risk of pathogenic organisms in higher parity groups, with a decrease in Lactobacillus spp. and overall microbiota diversity.

The largest proportion of women are grand multiparous (3+), comprising 30% (43 women) of the total. Primiparous women (first-time mothers) account for 25% (36 women), while multiparous women (having had 2 previous pregnancies) make up 26% (37 women). Nulliparous women (first pregnancy) represent 19% (28 women). This distribution highlights a diverse maternal demographic, with a significant number of women experiencing their third or subsequent pregnancies.

Discussion

Prevalence of vaginal microbiota among pregnant mothers attending Nakaseke Hospital

The study revealed that 55.6% of vaginal samples from pregnant women harbored detectable microbiota, with *Candida albicans* being the most prevalent organism at 15.2%, followed closely by *Gardnerella vaginalis*, *Escherichia coli*, and *Streptococcus agalactiae*. Nearly half (44.4%) of the samples showed no detectable organisms, which aligns with findings by Romero et al. (2023), indicating that a substantial proportion of pregnant women may have a predominantly Lactobacillus-dominant microbiota or low microbial load during pregnancy. The high prevalence of *Candida albicans* suggests that fungal colonization is common during pregnancy, potentially influenced by hormonal changes that favor fungal overgrowth (Auriemma et al., 2021). The diversity of organisms identified underscores the complexity of vaginal

microbial ecology and its potential implications for maternal health and pregnancy outcomes.

Microbial diversity present in the vaginal samples among pregnant women at Nakaseke Hospital

The study revealed a diverse vaginal microbiota among pregnant women, with *Candida albicans* being the most prevalent organism, detected in 15.2% of participants. Notably, 44.4% of samples showed no detectable organism, indicating a substantial proportion of women with an apparently sterile or undetectable microbiota. The presence of *Gardnerella vaginalis*, *Escherichia coli*, and *Streptococcus agalactiae* at notable frequencies suggests potential dysbiosis and risk for infections such as bacterial vaginosis and neonatal group B streptococcus transmission (Ainomugisha et al., 2023; Baud et al., 2023). These findings underscore the complex microbial landscape during pregnancy, aligning with previous research indicating that vaginal microbiota varies significantly among women and impacts pregnancy outcomes (Romero et al., 2023; Auriemma et al., 2021).

Relationship between maternal age and vaginal microbiota composition among pregnant women at Nakaseke Hospital

The analysis demonstrated age-related variations in vaginal microbiota composition. Younger women (18-24 years) predominantly harbored Lactobacillus spp., associated with vaginal health and protection against infections (Auriemma et al., 2021). Conversely, older women (35+) showed a higher prevalence of *Candida albicans* (80%) and absence of Lactobacillus, suggesting increased susceptibility to fungal infections or microbiota shifts with age (Shabayek et al., 2022). The 30-34 age group exhibited increased *Gardnerella vaginalis* and *Streptococcus agalactiae*, indicating a higher risk of bacterial vaginosis and GBS colonization, which could influence pregnancy outcomes (Hashiramoto et al., 2023). These findings support the notion that maternal age influences vaginal microbial composition, with potential implications for maternal and neonatal health.

Relationship between maternal parity and vaginal microbiota composition among pregnant women at Nakaseke Hospital

Parity appeared to influence vaginal microbiota diversity. Primiparous women had a higher proportion of Lactobacillus spp., suggesting a more protective microbiota profile. With increasing parity, there was a noticeable rise in *Candida albicans*, *Gardnerella vaginalis*, and *Streptococcus agalactiae*, indicating a shift toward dysbiosis and increased colonization by potential pathogens (Mulinganya et al., 2021; Romero et al., 2023). Women with three or more previous births showed the highest prevalence of *Candida albicans* and *Streptococcus agalactiae*, which could elevate the risk of maternal infections and adverse neonatal outcomes, aligning with findings from other studies on parity-related microbiota changes (Fardi et al., 2024).

Conclusion

The findings revealed that over half of the pregnant women harbored detectable microbiota. The study highlights significant variations in vaginal microbiota among pregnant women at Nakaseke Hospital, influenced by maternal age and parity. Younger women tend to have Lactobacillus-dominant microbiota, which is protective, whereas older and multiparous women exhibit increased colonization by pathogenic organisms such as *Candida albicans*, *Gardnerella vaginalis*, and *Streptococcus agalactiae*. These microbiota shifts could predispose women to infections like bacterial vaginosis, candidiasis, and GBS-related complications, potentially impacting maternal and neonatal health outcomes.

Recommendation

Implement screening for vaginal microbiota composition during pregnancy, especially in women of advanced age and higher parity, to identify dysbiosis early.

Promote probiotic interventions or microbiota modulation strategies to enhance Lactobacillus colonization and reduce pathogenic organisms, thereby improving pregnancy outcomes.

Conduct longitudinal studies to understand the dynamics of vaginal microbiota throughout pregnancy and its direct impact on maternal and neonatal health, incorporating molecular techniques for comprehensive microbial profiling.

Educate pregnant women on the importance of vaginal health and hygiene practices tailored to age and parity-related risks.

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List of abbreviations

UGS: Uganda Government Standards
WHO: World Health Organization
ANC: Antenatal Care
UTIs: Urinary Tract Infections
BV: Bacterial Vaginosis
STIs: Sexually Transmitted Infections
LMP: Last Menstrual Period

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The study was not funded.

Conflict of interest

The author declares that there was no conflict of interest.

Author contributions

SN- Developed and investigated the study.
HN- Supervised the study.
TG- Supervised the study.
FS- Supervised the study.

Data availability

Data is available upon request.

Informed consent

Written informed consent was obtained from all participants prior to their inclusion in the study. Participants were informed about the purpose of the study, procedures involved, potential risks and benefits, and their right to withdraw at any time without penalty.

Author biography

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