Vulval lichen sclerosus and the microbiome

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INTRODUCTION

Female genital lichen sclerosus (FGLSc) is a chronic, scarring inflammatory dermatosis associated with significant morbidity and squamous cell carcinoma. To date, role of the microbiome in FGLSc has received limited attention.

HYPOTHESIS

It is hypothesised that dysbiosis plays a role in the aetiopathogenesis of female genital lichen sclerosus (FGLSc).

METHODS

Case-control study with follow-up
- 30 patients with a clinical (+/- histological) diagnosis of untreated, active FGLSc and 30 healthy controls (no genital dermatosis)
- Recruitment: September 2022 – March 2024

Inclusion: Female patients aged ≥18
Exclusion:
- Immunocompromise (e.g., diabetes, malignancy)
- Recent infection/systemic antibiotics within 4 weeks
- Genital topical steroid/antibiotic use within 6 weeks
- Significant inflammatory dermatosis elsewhere

Samples:
- Vaginal swab (mid vagina)
- Vulval swab (labia majora, minora, interlabial sulcus)
- First-catch urine

Samples taken at baseline and three months. During this period, cases were prescribed clobetasol propionate 0.05% ointment: daily for 1 month, alternate days for 1 month, then twice weekly for 1 month.

Analysis: 16S rRNA next-generation sequencing

RESULTS

Baseline characteristics of cases and controls were comparable (Table 1). A reduction in alpha diversity was seen in patients with FGLSc at all three sites compared with controls, with a statistically significant difference noted in vulval samples (p=0.0415) (Fig 1).

Table 1: Baseline characteristics of cases and controls

Characteristic	FGLSc group Median (IQR) or n (%)	Control group Median (IQR) or n (%)	P-value	Characteristic	FGLSc group Median (IQR) or n (%)	Control group Median (IQR) or n (%) (	P-value
Age (years)	58 (48.25-66.75)	53.5 (41.75-64.5)	0.39	HRT	6 (20%)	6 (20%)	>0.99
BMI	25.15 (22.6-29.25)	24 (20.5-24.65)	0.19	Hysterectomy	3 (10%)	2 (6.7%)	>0.99
Parity	2 (0-2.25)	1.5(0-2)	0.45	Smoker	4 (13.3%)	0 (0%)	0.11
Post-menopausal	18 (60%)	18 (60%)	>0.99	Washing frequency	1 (1-1)	1 (1-1)	0.72

Figure 1: Alpha diversity of baseline samples

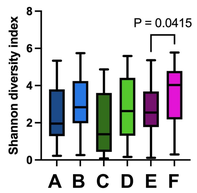

Figure 1 Shannon diversity index comparing microbiota between patients with FGLSc and healthy controls across urine cases (A), urine controls (B), vaginal cases (C), vaginal controls (D), vulval cases (E), and vulval controls (F).

This index reflects both the richness and evenness of bacterial amplicon sequence variants.

Beta diversity was measured using Bray-Curtis distance, with groups compared using PERMANOVA analysis. A significant difference was observed at baseline between cases and controls at vulval (pseudo-F 2.25, p=0.001), vaginal (pseudo-F 1.69, p=0.013) and urine (pseudo-F 1.62, p=0.028) sites at baseline. There was no significant difference in alpha or beta diversity before and after treatment of FGLSc.

Vaginal microbiome at baseline
At baseline, the dominant bacterial genera in both cases and controls were *Lactobacillus spp.*, *Streptococcus spp.*, *Gardnerella spp.*, and *Prevotella spp.* (in order of prevalence) (Fig 2). LEfSe (Linear discriminant analysis Effect Size) determines the organisms most likely to explain differences between groups; this identified a depletion of *Streptococcus spp.*, *Corynebacterium spp.*, *Fenollaria spp.*, *Alloscardovia spp.*, *Escherichia-Shigella spp.*, *Propionibacterium spp.*, *Lawsonella spp.*, and *Pseudoglutamicibacter spp.* in cases compared with controls (Fig 3).

Vulval microbiome
In cases, the most common bacteria at baseline (in order of prevalence) were *Lactobacillus spp.*, *Streptococcus spp.*, *Gardnerella spp.*, and *Prevotella spp.*, while in controls, *Finegoldia spp.* replaced *Gardnerella spp.* in this list (Fig 2). LEfSe analysis showed enrichment of *Bifidobacterium spp.* and depletion of *Streptococcus spp.*, *Mobiluncus spp.*, *Actinomyces spp.*, *Jonquetella spp.*, *Lawsonella spp.*, *Saccharimonadales spp.*, and *Propionibacterium spp.* in cases relative to controls (Fig 3).

Urinary microbiome
At baseline, the most prevalent bacteria in cases were *Lactobacillus spp.*, *Streptococcus spp.*, *Gardnerella spp.*, and *Prevotella spp.*, whereas in controls, *Prevotella spp.* ranked higher than *Gardnerella spp.* (Fig 2). LEfSe analysis revealed enrichment (LDA log score >2) of *Escherichia-Shigella spp.* and *Jonquetella spp.*, alongside depletion of *Saccharimonadales spp.*, *Mobiluncus spp.*, and *Streptococcus spp.* in cases compared with controls (Fig 3)

Figure 2: Baseline composition of microbiota

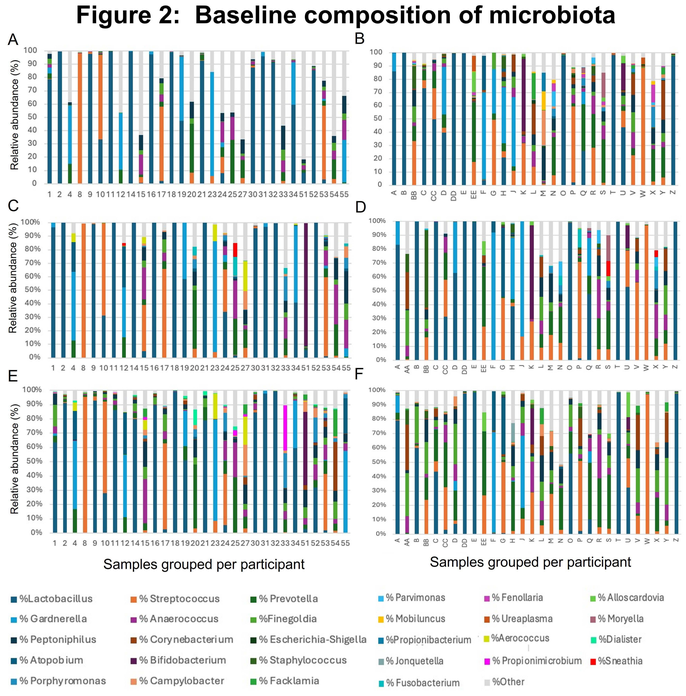

Figure 2 Relative abundance of microbiota at the genus level for baseline FGLSc cases (A) versus healthy controls (B), vulval cases (C) versus controls (D), and urinary cases (E) versus controls (F)

Changes in microbiota following treatment
Following treatment of FGLSc, no significant changes in vaginal microbiota composition were detected by LEfSe analysis. In contrast, untreated controls exhibited a reduction in *Gardnerella* spp., *Bifidobacterium* spp., *Dialister* spp., and spp.

Changes in microbiota following treatment continued
At the vulval site, treated cases showed an enrichment of *Fastidiosipila* spp., whereas untreated controls demonstrated a depletion of *Actinotignum* spp., *Jonquetella* spp., *Dialister* spp., and Gardnerella spp. compared with their baseline samples.

In urine samples, treated cases displayed a decrease in *Bacteroides* spp. and *Ezakiella* spp. In untreated controls, there was an enrichment of *Xanthomonas* spp. and *Howardella* spp., along with a depletion of *Mobiluncus* spp., *Dialister* spp., and *Solobacterium* spp. relative to baseline.

Figure 3: Differences in baseline microbiota composition

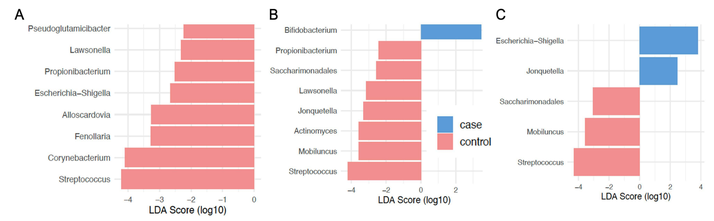

Figure 3 LEfSe analysis illustrating variations in microbiota composition between women with FGLSc and controls, at the vagina (A), vulva (B), and urine (C) (LDA score > 2.0, p < 0.05). The findings are displayed through bar plots, showing the LDA scores for each genus.

CONCLUSIONS

- Reduction in alpha diversity in **patients with FGLSc at vaginal, vulval and urinary sites is indicative of reduced richness and evenness** of species. This has been previously reported in vulval swabs from patients with FGLSc[1].
- The **overall microbial communities differed between cases and controls at vaginal, vulval and urinary sites**, as evidenced by the statistically significant difference in beta diversity.
- Across the vaginal, vulval, and urinary microbiomes, ***Lactobacillus* spp. was the most prevalent genus overall at baseline in both cases and controls**, consistent with its established role in maintaining urogenital health.
- Several differences in less abundant microbiota were identified within the vaginal, vulval and urinary microbiota when patients with FGLSc were compared with controls. These findings suggest that **dysbiosis may contribute to the aetiopathogenesis of FGLSc.**
- **No changes in vaginal microbiota composition were observed following FGLSc treatment.** While some alterations were detected at the vulval and urinary sites, other variations were also identified in follow-up samples from untreated controls across all three sites when compared with baseline samples.
- Key strengths of this study included the **recruitment of patients with active, untreated FGLSc**, the use of stringent exclusion criteria to **minimise the impact of confounding factors** on microbial composition, and a **high level of comparability between case and control groups** across numerous variables known to influence the microbiome. The study is limited by a relatively small sample size and 16S ribosomal RNA sequencing limiting species-level classification.

REFERENCES

[1]Pyle HJ, Evans JC, Artami M, Raj P, Sridharan S, Arana C, Eckert KM, McDonald JG, Harris-Tryon TA, Mauskar MM. Assessment of the Cutaneous Hormone Landscapes and Microbiomes in Vulvar Lichen Sclerosus. *J Invest Dermatol.* 2024 Aug;144(8):1808-1816.e11

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