Original Report

Clinical and Microbiological Findings of Vulvovaginitis in Prepubertal Girls

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A B S T R A C T

Study Objective: To evaluate genital microbiological findings in prepubertal girls with vulvovaginitis and in healthy controls.
Design: Prospective case-control study.
Setting: Pediatric Outpatient unit of the Department of Pediatrics of the Hospital of the Lithuanian University of Health Sciences Kauno Klinikos from November 2014 to May 2017.
Participants: Fifty-two prepubertal girls aged 1-9 years diagnosed with vulvovaginitis, and 42 age-matched healthy controls.
Interventions and Main Outcome Measures: Samples for microbiological culture were collected using sterile cotton swabs from the introitus and the lower third of the vagina from all study participants. Microbiological findings were analyzed according to bacteria type and intensity of growth.
Results: Most of the vaginal microbiological swab results were positive for bacterial growth: 47 (90.4%) and 34 (80.9%) were similar in the study and control groups, respectively ($P = .24$). Sixteen (30.8%) and 9 (21.4%) of the microbiological traits results in the case and control groups, respectively, were regarded as potential causative agents ($P = .27$). *Streptococcus pyogenes* was the most frequent pathogen in the study group ($P = .03$); all other microorganisms detected as either a pure or dominant growth in the control group, were considered opportunistic.
Conclusions: Vaginal bacterial culture results were positive in prepubertal girls with vulvovaginitis and in healthy controls. Nonspecific vulvovaginitis without a dominant/isolated pathogen was seen to be more common than vulvovaginitis with a potential causative agent. Clinical symptoms were more frequent among girls when the potential infectious agent was identified.
Key Words: Prepubertal girls, Vulvovaginitis, Microbiological traits, Clinical symptoms

Introduction

Vulvovaginitis is a common gynecological problem in prepubertal girls and frequently causes anxiety in the child and her parents. Prepubertal girls are at increased risk for vulvovaginal inflammation because of anatomic, physiological, and behavioral factors.[1–4]

The diagnosis of vulvovaginitis in prepubertal girls is usually on the basis of clinical history and inspection of the external genitalia.[1,2] The microscopic evaluation and culture of vulvovaginal secretions are considered additional tools in routine clinical practice.[3,4]

On the basis of microbiological data, most cases of vulvovaginitis are considered to be nonspecific, because mixed growth cultures are seen more often than an isolated pathogen from most of the vaginal swabs obtained.[4–6] Notably, because there are few studies on normal vaginal microflora in healthy prepubertal girls, it is difficult to determine whether an isolated type of bacterium can be the causative agent of vulvovaginal inflammation.[5] Most opportunistic pathogens can be considered to form part of the normal vaginal microflora in prepubertal girls. Therefore, vaginal bacterial cultures from patients with vulvovaginitis should be evaluated cautiously when deciding the cause of inflammation and considering specific antibacterial management.[3,4]

In our study we aimed to evaluate microbiological findings in prepubertal girls with vulvovaginitis compared with those in healthy controls.

Materials and Methods

A prospective case-control study was carried out from November 2014 to May 2017 at the Pediatric Outpatient unit in the Department of Pediatrics at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos. Prepubertal girls with a suspected diagnosis of vulvovaginitis were eligible for the study. All patients underwent routine evaluation including, history, visual inspection of the vulvovaginal area, and bacteriological sampling as outlined below. The diagnosis of vulvovaginitis was on the basis of the following symptoms and signs such as pain, itching, burning, soreness, dysuria, rash, white plaques, inflammation, and (or) discharge.

The authors indicate no conflicts of interest.

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Patients with Tanner stage I pubertal development and a diagnosis of vulvovaginitis were included in the study. Patients with a history of suspected sexual abuse or a suspected vaginal foreign body were excluded from the study.

The consecutive age-matched and Tanner stage I healthy girls examined for routine screening during the study period at the Pediatric Outpatient unit were invited to participate in the study as controls. Girls with no symptoms or signs of vulvovaginitis, other infectious diseases, no use of antibiotics during the past 6 months, and no immunosuppressive illnesses, were recruited after obtaining written informed consent from the patient and her parents/guardians for the control group.

The patients were examined in the "frog-leg" position, assisted by the child's parents/guardians. Samples for microbiological culture were collected using sterile cotton swabs from the introitus and the lower third of the vagina, and swabs were immediately placed into Amies Transport Medium (Becton Dickinson), stored, and sent within 24 hours to the microbiology laboratory. Samples were inoculated directly onto 5% sheep blood agar (BBL), chocolate agar (BBL), and MacConkey agar plates (Oxoid). Sheep blood and chocolate agar plates were incubated at 35°C in an atmosphere containing 5% CO_2 and MacConkey agar plates (Oxoid) at 35°C for 18-24 hours. In cases of a negative culture result at the first observation, sheep blood (BBL) and chocolate agar (BBL) plates were reexamined after an additional 24 hours of incubation. Isolated microorganisms were identified using the Maldi-Tof ID system (Bruker).

The microbiological findings were classified into several groups according to the bacterial growth intensity. Specimens with no bacterial growth were considered negative. Specimens were assessed as positive when pure or mixed cultures were identified. Specimens with a single isolated bacterium growing in culture were considered pure, whereas specimens with more than 1 bacterium identified were considered mixed. According to the growth intensity on a Petri dish, specimens were classified as follows: group I, sporadic or medium growth (1 or 2 quadrants filled with growing bacterial culture); group II, high or very high growth (3 or 4 quadrants filled with bacterial culture). Pure culture or dominant microorganisms in a mixed culture were considered to be potential causative agents of inflammation, whereas other mixed culture findings were deemed to be normal vaginal microflora. The microorganisms detected were classified as pathogens, opportunistic pathogens, and nonpathogens (Fig. 1).

Table 1
Distribution of Age in the Study Group and the Control Group (N = 94)

Age, years	Study Group	Control Group
1	2 (3.8)	6 (14.3)
2	4 (7.7)	5 (11.9)
3	11 (21.2)	10 (23.8)
4	10 (19.2)	5 (11.9)
5	7 (13.5)	7 (16.7)
6	10 (19.2)	4 (9.5)
7	2 (3.8)	2 (4.8)
8	2 (3.8)	3 (7.1)
9	4 (7.7)	0 (0.0)
Mean (SD)	4.5 (2.2)	3.9 (2.1)

Data are presented as n (%) except where otherwise noted.

Processing of the statistical data and establishing statistical significance was performed using the χ^2 test, and statistical analysis was performed using SPSS Statistics version 23 (IBM Corp) and Microsoft Excel 2010. The degree of error set below 5% was deemed to be the threshold of statistical significance ($P < .05$).

The study was approved by the Lithuanian Bioethics Committee. The written informed consent form for participation was signed by the parents/guardians of all study participants.

Results

The study group was composed of 52 prepubertal girls aged 1-9 (mean, 4.5 ± 2.2) years, and 42 healthy girls with a mean age of 3.9 (±2.1) years in the healthy control group. The difference in mean age in the study and the control groups was not statistically significant. The mean (SD) age

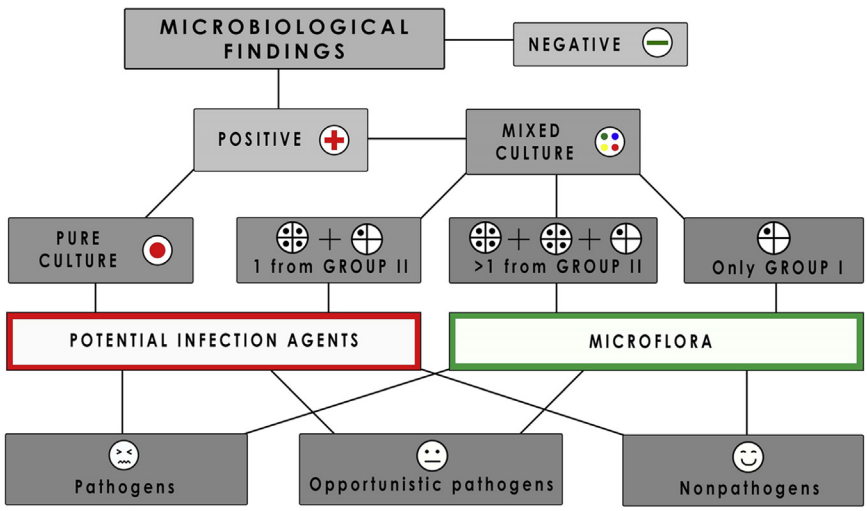

Fig. 1. Distribution of microbiological findings according to bacterial growth intensities. The results of vaginal introitus and lower third of vagina swab were classified into 2 groups: group I, sporadic/medium growth; and group II, high/very high growth.

Table 2
Clinical Symptoms of Vulvovaginitis in Correlation with the Presence of Potential Infection Agents in Vaginal Swabs

Clinical Symptom	Potential Infection Agent (n = 16)		Microflora (n = 36)		*P*	Total Number of Patients (n = 52)	
	n	%	n	%		n	%
Vaginal discharge	16	100.0	18	50.0	.06	34	65.4
Genital redness	9	56.2	23	63.9	.19	32	61.5
Itch	8	50.0	12	33.3	.33	22	42.3
Pain	8	50.0	10	27.8	.31	18	34.6
Burning	5	31.3	6	16.7	.50	11	21.2
White plaque	5	31.3	4	11.1	.32	9	17.3
Rash	4	25.0	3	8.3	.48	7	13.5
Soreness	4	25.0	2	5.6	.22	6	11.5
Dysuria	2	12.5	4	11.1	.58	6	11.5

of all study participants was 4.2 (±2.0) years (range, 1-9 years). In the study group, 52 girls had clinically diagnosed vulvovaginitis, in 38 (73%) cases, girls were 3-6 years old (Table 1).

The most frequent clinical symptoms of vulvovaginitis were vaginal discharge 34/52 (65.4%), redness 32/52 (61.5%), itching 22/52 (42.3%), and pain 18/52 (34.6%; Table 2).

Forty-seven of 52 (90.4%) and 34/42 (80.9%) of all vaginal culture results were positive for bacterial growth in study and control groups, respectively ($P = .24$; Table 3). Potential causative bacteria were equally distributed in both groups, with 16/52 (30.8%) cases in the study group and 9/42 (21.4%) in the control group ($P = .27$). The number of bacteria considered to be vaginal microflora exceeded the number of potential causative agents in both groups ($P = .04$; Table 3).

Streptococcus pyogenes was the most frequent pathogen in the study group (n = 9/16 (56.2%); $P = .03$; Table 4), seen in pure culture samples; in 5 cases, *Streptococcus pyogenes* was seen as low-intensity growth in mixed culture. All other dominant microorganisms in the control group were opportunistic, the most frequent being *Enterococcus fecalis* and *Staphylococcus aureus* ($P = .09$; Table 4). Vaginal microflora were similar in both groups; enteric and skin bacteria, dominance of *Escherichia coli, Enterococcus fecalis,* and *Enterococcus epidermidis*, being most frequent. There was no statistical difference observed between the types of bacteria in the study and the control groups (Fig. 2).

Although not statistically significant, clinical symptoms were more frequent among girls in the presence of a potentially infectious agent, except for the symptoms of genital redness and dysuria, the frequency of which was similar in both groups (Table 2).

Discussion

Vaginal microflora is dependent on pubertal status. In prepubertal girls, *Lactobacilli*, *Diphteroids*, and *Gardnerella Vaginalis* are usually absent, which permits pathogenic and opportunistic bacteria to alter the vaginal epithelium, causing vulvovaginitis.[4,6,7] Thus the presence of opportunistic bacteria in vaginal swab culture during vulvovaginitis is of clinical relevance. In this study, opportunistic pathogens as a pure culture were detected in 7 symptomatic girls, and specific pathogens were identified in 9 (Table 4).

In accord with earlier reports, vaginal discharge and genital redness, itching, and pain were the most frequent complaints in symptomatic girls with vulvovaginitis.[4,8–12] Similarly, it was difficult to relate the clinical features of vulvovaginitis to specific pathogens that might be causing this illness, but the manifestation of the disease was more acute when individual or dominant bacteria were found.[7,8]

Vulvovaginitis in prepubertal girls has been reported as nonspecific in 25%-75% of cases,[2,6,9,12] as was also the case in the present study, in 59.6% of symptomatic girls. Consequently, the vaginal swab culture results should be evaluated with great caution because the bacteria detected could simply represent normal vaginal microflora (Table 3). Specific treatment for vulvovaginitis is indicated when an isolated high-growing bacterium is detected in pure culture.[1,3] With respect to mixed culture samples, some authors have reported that dominant and high-growing microorganisms should also be considered as potential causative agents of inflammation and as such justify treatment.[5] In contrast our data suggest that because isolated or dominant bacteria are also abundantly present in the control group, the severity of the symptoms should guide the management strategy.

Table 3
Vaginal Microbiological Swab Results (n = 94)

Variable	Study Group (n = 52)		Control Group (n = 42)		*P*
	n	%	n	%	
Pure culture	10	19.2	4	9.5	.15
Mixed culture					
1 From group II	6	11.5	5	11.9	.60
More than 1 from group II	6	11.5	9	21.4	.15
Only group I	25	48.1	16	38.1	.47
Negative	5	9.6	8	19.1	.16
Potential infection agent	16	30.8	9	21.4	.27
Microflora	36	69.2	33	78.6	.04

Table 4
Distribution of Microorganisms in Girls with Vulvovaginitis and Healthy Controls (n = 94)

Microorganism	Study Group (n = 52)			Control Group (n = 42)			*P*
	Potential Infection Agent	Microflora	Total Number (%)	Potential Infection Agent	Microflora	Total Number (%)	
Specific pathogen							
Streptococcus pyogenes	9	5	14 (26.9)	0	1	1 (2.3)	.001
Haemophilus influenzae	0	1	1 (1.9)	0	0	0 (0.00)	.55
Opportunistic pathogens							
Escherichia coli	1	18	19 (36.5)	1	20	21 (50.0)	.14
Enterococcus faecalis	0	12	11 (21.1)	2	6	8 (19.0)	.42
Proteus mirabilis	0	1	1 (1.9)	0	0	0 (0.0)	.55
Staphylococcus aureus	1	4	5 (9.6)	2	1	3 (7.1)	.48
Streptococcus pneumoniae	0	1	1 (1.9)	0	0	0 (0.0)	.55
α-hemolytic streptococcus	0	1	1 (1.9)	0	1	1 (2.3)	.48
β-hemolytic streptococcus	1	3	4 (7.7)	1	2	2 (4.7)	.62
Corynebacterium amycolatum	4	2	6 (11.5)	1	5	6 (14.2)	.48
Candida albicans	0	1	1 (1.9)	0	2	2 (4.7)	.42
Nonpathogens							
Fecal flora							
Escherichia hermannii	0	0	0 (0.0)	0	2	2 (4.7)	.20
Salmonella enterica	0	1	1 (1.9)	0	0	0 (0.0)	.55
Klebsiella oxytoca	0	1	1 (1.9)	0	1	1 (2.3)	.48
Citrobacter freundii	0	0	0 (0.0)	0	3	3 (7.1)	.09
Raoultella planticola	0	1	1 (1.9)	0	0	0 (0.0)	.55
Skin flora							
Staphylococcus epidermidis	0	10	10 (19.2)	0	7	7 (16.6)	.48
Staphylococcus hominis	0	2	2 (3.8)	1	1	2 (4.7)	.61
Staphylococcus capitis	0	0	0 (0.0)	0	1	1 (2.3)	.48
Staphylococcus simulans	0	1	1 (1.9)	0	1	1 (2.3)	.48
Staphylococcus warneri	0	1	1 (1.9)	0	0	0 (0.0)	.55
PNS	0	1	1 (1.9)	0	1	1 (2.3)	.48
Micrococcus luteus	0	0	0 (0.0)	0	1	1 (2.3)	.45
Staphylococcus haemolyticus	0	5	5 (9.6)	0	3	3 (7.1)	.48
Acinetobacter lwoffii	0	0	0 (0.0)	0	1	1 (2.3)	.45
Mouth flora							
Streptococcus oralis	0	2	2 (3.8)	0	0	0 (0.0)	.30
Streptococcus mitis	0	1	1 (1.9)	1	0	1 (2.3)	.48
Neisseria mucosa	0	0	0 (0.0)	0	1	1 (2.3)	.45
Neisseria perflava	0	0	0 (0.0)	0	2	2 (4.7)	.20
Nasopharyngeal flora							
Streptococcus anginosus	0	5	5 (9.5)	0	3	3 (7.1)	.48
Streptococcus constellatus	0	1	1 (1.9)	0	0	0 (0.0)	.55
Pseudomonas monteilii	0	0	0 (0.0)	0	1	1 (2.3)	.45

Negative culture results were found only in 19.1% of healthy control participants (Table 3).

Streptococcus pyogenes is the most commonly identified pathogen in girls with vulvovaginitis,[2,8,11,13] seen accompanying or after symptomatic pharyngitis at times. *Streptococcus pyogenes* was isolated from 8%-47% of cases in prepubertal girls with vulvovaginitis,[2,5] consistent with our findings, 17.3% in the study group, but only once in the

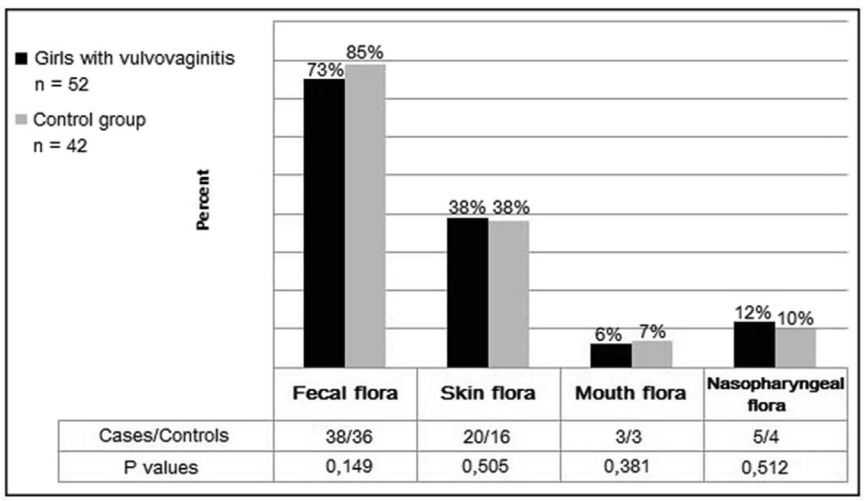

Fig. 2. Distribution of bacteria types in the study and the control groups (N = 94).

control group, thus considered to be of the normal microflora and sporadic growth (Table 4).

The course of vulvovaginitis caused by *Streptococcus pyogenes* is generally acute with seropurulent or blood-stained discharge, erythema, including the perineal area, burning skin, and dysuria.[3] Abnormal purulent, thick discharge, and perineal erythema were detected in all study group girls associated with a pure culture of *Streptococcus pyogenes*.

Haemophilus influenza has been reported as the second most frequently isolated pathogen causing vulvovaginitis among prepubertal girls.[10] The low frequency of *Haemophilus influenza* in our study could be explained by the *Haemophilus influenza* type b vaccination, which has been available in Lithuania since 2004,[8,14] ensuring protection against respiratory tract infections as well as vulvovaginitis.

Candida albicans is a rare finding in the genitalia of prepubertal girls[2,5,12] and was detected only once in the study group and twice among control participants with low growth intensity. Because many clinicians still believe in a fungal origin of vulvovaginitis among prepubertal girls, there remains excessive use of antifungal agents, increasing the risk of resistance.[6,15]

The causal relationship between *Staphylococcus aureus* and vulvovaginitis remains controversial, in accord with our findings and other studies[2,5,13] (Table 4). The data from other studies revealed that *Staphylococcus aureus* was excluded in 2.5%-7% of the patients.[5,7,14] *Staphylococcus aureus* was detected in 3 (3.2%) girls in our study, only 1 (1.06%) of whom had symptoms of vulvovaginitis. Mostly, *Staphylococcus aureus* was revealed in mixed culture and was not considered the main pathogen.

The role of fecal bacteria in the pathogenesis of this condition is still unclear. *Escherichia coli* and *Enterococcus fecalis* are usually the most common opportunistic pathogens in girls with abnormal discharge.[11,16] Randelovic et al[2] reported bacteria of fecal origin in one-third of symptomatic girls; in almost all cases, pure cultures were isolated. In contrast, we detected fecal flora in 61.5% of girls in the study group; dominance was seen only once (Fig. 2). However, the frequent presence of fecal flora in our cultures of healthy prepubertal girls is in agreement with previous findings.[2,3,10,12] We believe that the frequent detection of fecal flora in vaginal cultures could be explained by poor hygiene, the anatomical proximity of the vulva to the anus, and the ability of fecal flora to survive in different pH environments.

Hammerschlag et al[17] reported a high prevalence of diphtheroids, *Staphylococcus epidermidis,* and enteric bacteria in the cultures of healthy prepubertal girls. The same vaginal microflora in healthy and symptomatic girls with vulvovaginitis were reported by Jaquery et al,[18] consistent with our findings. We found the same microflora in the study and control groups with a prevalence of skin and gastrointestinal bacteria (Fig. 2). The prevalence of these types of bacteria is caused by their dissemination in the whole body and high viability.[7]

Our study had several limitations. We had difficulties in recruiting the control group because of social and cultural perceptions, as well as persuading parents/guardians to provide consent for interventions in healthy girls. In future studies a twofold increase in the number of subjects in the control group would ensure more precise results and higher statistical power.

Conclusions

Our findings suggest that positive vaginal bacterial culture results are detected in girls with symptomatic vulvovaginitis and in healthy control participants. Mixed vaginal culture without a dominant/isolated pathogen was found in most cases. There was no difference in the types of bacteria identified in symptomatic and healthy girls. The most common pathogen of vulvovaginitis was *Streptococcus pyogenes*. Clinical symptoms were more frequent among girls when the potential infectious agent was detected. Our study revealed a nonspecific origin of vulvovaginitis in most prepubertal girls.

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