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ORIGINAL ARTICLE

Characteristics of Vulvo-Vaginal Infections in 9- to 13-Year-Old Girls Undergoing Rapid Puberty

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SUMMARY

Background: **This study was conducted to investigate the characteristics of vulvo-vaginal infections in 9- to 13-year-old girls undergoing rapid puberty.**
Methods: **Three hundred ninety girls aged 9 - 13 years who experienced vulvo-vaginal infections while undergoing rapid puberty and were treated at West China Second University Hospital from July 2017 to June 2020 were retrospectively analyzed. The incidences of bacterial vaginosis (BV), intermediate BV, and vulvo-vaginal candidiasis (VVC) and the differences in these incidences for patients of different ages were analyzed.**
Results: **The incidences of BV, intermediate BV, VVC, and unknown pathogenic vaginitis were 35.38%, 35.13%, 19.23%, and 10.26%, respectively. The incidence of BV was significantly higher than that of VVC. The positive rates of *Candida albicans (C. albicans)* and *non-albicans Candida* infections differed significantly at 80.00% and 20.00%, respectively. The BV and intermediate BV incidences did not significantly differ by age. The VVC incidence was significantly lower for 9-year-old girls than for girls of other ages.**
Conclusions: **Girls undergoing rapid puberty are more susceptible to BV and intermediate BV infections than to VVC infections. The VVC incidence was lowest in 9-year-old girls. More attention should be paid to the effects of female estrogen levels, the vaginal microecosystem, and menstrual hygiene on vulvo-vaginal infections in girls undergoing rapid puberty.**
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KEY WORDS

rapid puberty, adolescent development, bacterial vaginosis, vulvo-vaginal infection

INTRODUCTION

Vulvo-vaginal infections are common gynecological diseases [1]. The physical conditions of the female genital tract are significant for female growth, both physically and psychologically [2]. Puberty is the key developmental period from immaturity to maturity for the female reproductive system. Significant changes in physical growth, gonadal development, secondary sexual characteristics, and psychological behavior occur during puberty [3]. Puberty is divided into three stages: early puberty (6 - 8 years old), rapid puberty (9 - 13 years

old), and late puberty (14 - 18 years old) [4]. Rapid puberty is a key and sensitive period for girls' physical and mental development. Studies have shown that in China, girls undergoing rapid puberty lack knowledge of how to prevent genital diseases during puberty [2]. Girls undergoing rapid puberty tend to have poorer physical hygiene than do girls in early and late puberty [5]. The health condition of the genital tract directly affects girls' physical and mental development [6]. For girls undergoing rapid puberty, the reproductive organs and functions mature quickly, estrogen secretion levels are relatively high, and the amount of vaginal discharge is high. Vulvo-vaginal infections in girls undergoing rapid puberty are mainly due to poor hygiene, decreased immunity, and a lack of parental care and knowledge of reproductive health [7]. Therefore, the conditions and differences in vulvo-vaginal infections for the reproductive health of girls undergoing rapid puberty must be investigated. Vulvo-vaginal candidiasis (VVC) is one of the most important types of vulvo-vaginal infections and is caused by *Candida* species when immunoactivity is lower either in the vagina or systemically for girls undergoing rapid puberty. This study was conducted to determine the characteristics of vulvo-vaginal infections in 9 - to 13-year-old girls undergoing rapid puberty and the differences and incidences of these infections.

MATERIALS AND METHODS

Patients

Three hundred ninety girls aged 9 - 13 years who were treated at West China Second University Hospital, Sichuan University, from July 2017 to June 2020 were divided into five age groups of 9, 10, 11, 12 and 13 years old. All participants and their guardians provided written informed consent.

Inclusion criteria were as follows. 1) All participants were girls undergoing rapid puberty and aged 9 - 13 years. 2) All participants had clinical symptoms of vulvar discomfort or vulvar redness and swelling, dysuria, pruritus vulvae, and abnormal vaginal discharge. 3) All participants had clinical symptoms of vulvar discomfort or were diagnosed with vulvo-vaginal infections but were not previously treated with anti-infective drugs for vaginitis.

Potential participants were excluded if they had been treated with anti-infective agents for vaginitis or other cleaning agents or pharmaceuticals that would interfere with the diagnosis, were reexamined in our hospital for the same condition (to exclude those with recurrent infections), were not undergoing rapid puberty despite being 9 - 13 years old.

Methods

For 9- to 13-year-old girls who were not sexually active, vaginal discharge samples were taken by inserting a sterile dry cotton swab into the vagina, then smearing the swab on sterile glass slides. A second sample was taken from the same location with another sterile dry cotton swab and placed in a culture tube containing sterile saline solution. (When small amounts of vaginal discharge were encountered, cotton swabs were moistened with sterile physiological saline to collect the material) [8]. The smears were heat-fixed, then Gram stained as per the *National Guide to Clinical Laboratory Procedures*. Cedarwood oil was added for 1,000 x oil-immersion microscopy to observe the morphological indicators of vaginal discharge, including leukocytes, epithelial cells, *Lactobacilli* spp., *Gardnerella vaginalis* and Gram variable curved *Mobiluncus* spp., *Trichomonas vaginalis*, *Candida* species, and gram-negative cocci [9]. Two experienced clinicians checked the results of all morphological tests. The collected vaginal discharge swabs were simultaneously sent to the microbiology laboratory for fungal culturing on Sabouraud-agar medium at 37°C for 24 - 96 hours, and positive fungal cultures were identified via VITEK mass spectrometry (Merieux, France).

Ethical considerations

All participants provided written informed consent, and their privacy rights were reserved. All procedures and protocols were performed in accordance with the Helsinki Declaration as revised in 2013. The Institutional Review Board of West China Second University Hospital, Sichuan University, approved the study protocol (Medical Research 2017, No. 25) on March 7, 2017 before the study began.

Criteria

Bacterial vaginosis (BV) and intermediate-type BV

After Gram staining of vaginal smears, the morphologic characteristic of bacteria and clue cells were observed under the microscope. The gram-positive rods were shown as suggestive for *Lactobacillus* spp. Gram-negative rods were shown as suggestive for *Gardnerella vaginalis.* The gram-variable rods were shown as suggestive for *Mobiluncus* spp. [10]. Clue cells are large, asymmetrical, mature vaginal epithelial cells with a large number of Gram-negative rods or gram-variable rods adhere to the cell surface, which make it is difficult to distinguish the edge of the epithelial cells. BV was diagnosed based on the Nugent scoring system, where scores of 4 - 6 were considered intermediate BV, and scores of 7 - 10 were considered BV (Table 1) [11]. BV was also diagnosed using the Amsel criteria, where presence of any three of the following four criteria was considered consistent with BV: 1) characteristic thin, homogenous vaginal discharge, 2) vaginal pH > 4.5, 3) release of a fishy amine odor after adding 10% KOH, and 4) clue cells constituting > 20% of the total epithelial cells [12]. In our study, the diagnosis of BV was based on the Nugent Scoring System.

Vulvo-vaginal candidiasis (VVC)

After Gram staining, *Candida* species appeared blue-purple [13]. Yeast without budding, yeast with budding,

and pseudohyphae were observed under an oil-immersion lens. *Candida* yeast appeared as gram-positive oval-shaped cells that were smaller than erythrocytes, with different lengths and diameters of 2 - 6 μm. Yeast with budding appeared as purple-black germ tubes of the protruding cell wall of *Candida* yeast forms. Pseudo hyphae appeared as extended germ tubes of the budding yeast [14]. VVC infection was diagnosed when yeast without budding, yeast with budding, and/or pseudohyphae were observed on the smears. Positive vaginal discharge was collected for culturing and identification, and VVC infection was divided into *Candida albicans* (*C. albicans*) *or non-albicans Candida (NAC)* infections.

Trichomoniasis

Trichomoniasis is a type of vaginitis caused by indirect or direct contact with exogenous Trichomonas vaginalis. The life cycle of Trichomonas vaginalis contains only trophozoites but no cysts. The living body of Trichomonas vaginalis is colorless and transparent, with refractive properties, changeable posture, and strong mobility. On Gram staining, Trichomonas vaginalis exhibit a larger volume than that of leukocytes, are 10 - 30 mm long, have diameters of 10 - 20 mm, and have a wide, pyriform, oval, or irregular shape [15]. T. vaginalis have a bubble-shaped nucleus at the front end of the body, with five matrixes arranged in rings that produce five flagella on top. These include four anterior flagella that are identical in length to the body and one posterior flagellum. The posterior flagellum is long and transparent and protrudes from the rear end by passing through the body. Trichomoniasis is diagnosed by Gram staining if Trichomonas vaginalis is detected.

Yeast colonization

When Gram staining the vaginal discharge samples revealed Candida yeast under oil-immersion microscopy, yeast colonization was further determined by culturing [16].

Statistical analysis

The vulvo-vaginal infection incidences are represented by percentages. The $\chi 2$ test was used to analyze differences in the incidences by age group and infection type. $p < 0.05$ was considered statistically significant [17].

RESULTS

Types and overall conditions of vulvo-vaginal infections in girls undergoing rapid puberty

We included 390 girls aged 9 - 13 years and undergoing rapid puberty in this study. The incidences of BV, intermediate BV, VVC, and unknown pathogenic vaginitis were 35.38% (138 patients), 35.13% (137 patients), 19.23% (75 patients, including 22 with mixed infections of BV, intermediate BV and VVC), and 10.26% (40 patients), respectively (Table 2). The incidence of fungal colonization was 0.51% (2 patients). No patients had trichomoniasis. Ten patients (2.56%) had mixed infections of BV and VVC; 12 patients (3.08%) had mixed infections of intermediate BV and VVC. All mixed infections included VVC; therefore, we considered all BV and intermediate BV infections as single infections. Of patients with VVC infections, 80.00% (60 patients) had *C. albicans* and 20.00% (15 patients) had *NAC*. The incidences of BV and intermediate BV did not significantly differ ($p = 0.9403$; Table 2), but both of these incidences differed significantly from that of VVC (both $p < 0.0001$).

Analysis of the differences between vulvo-vaginal infections and inflammation in girls at different ages

Four girls undergoing rapid puberty, the incidences of VVC differed significantly by age ($p = 0.0020$); however, the incidences of BV and intermediate BV did not (Table 3).

All patients were divided into five age groups of 9, 10, 11, 12, and 13 years old.

Multiple comparisons revealed that the VVC incidence was significantly lower in the 9-year-old girls than in the other age groups ($p < 0.0042$, < 0.0001, 0.0010, and 0.0032, respectively; Table 4).

DISCUSSION

Vulvo-vaginal infections are common gynecological diseases, with various infection types and conditions among people from different regions and of different ages [18]. Our study showed that girls undergoing rapid puberty were more susceptible to BV than to VVC infections. For VVC, the incidence of *C. albicans* was higher than that of *NAC*, and the incidence in 9-year-old girls was the lowest of all age groups.

BV infections are mixed infections of Gardnerella vaginalis and anaerobic bacteria and are usually caused by poor hygiene or indirect contact [19]. Studies have shown that various factors can cause BV infections, including estrogen levels, microbial diversity in the host vagina, environment, genetics and infection route [20-22]. VVC infection is a vulvo-vaginitis caused by Candida spp. when the immune response is lower either in the vagina or systemically or when the colonizing Candida exhibit an enhanced ability to adhere to vaginal epithelial cells [23]. Studies have shown that high estrogen and glycogen secretion levels can enhance the reproductive capacity of Candida spp., and the growth and reproduction of Candida spp. is enhanced in warm and humid environments [24]. Conversely, low estrogen levels are more conducive to BV and enhance the likelihood that BV will occur. The mechanism may be associated with a changing vaginal pH, vaginal microbial diversity, and immune status [25-27]. In our study, girls undergoing rapid puberty were significantly more susceptible to BV than to VVC. The main reason may be associated with estrogen levels in girls at this stage,

Table 1. Nugent scoring system for Gram staining.

Score	Lactobacillus spp.	Gardnerella vaginalis	Gram variable Mobiluncus spp.
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	0
4	0	4+	0

Scores and distributions were calculated by determining the average quantity of each bacterial type observed in every ten oil-immersion fields. 0: No bacteria in an oil-immersion field, 1+: < 1 bacterium per oil-immersion field, 2+: 1 - 4 bacteria per oil-immersion field, 3+: 5 - 30 bacteria per oil-immersion field, and 4+: > 30 bacteria per oil-immersion field. The total score was calculated by adding the three bacterial scores. Normal: 0 - 3, Intermediate BV: 4 – 6, BV: 7 - 10.

Table 2. Differences in the incidences of BV, intermediate BV and VVC in girls undergoing rapid puberty.

	BV(a)	intermediate type of BV(b)	VVC(c)	Unknown pathogenic vaginitis
n	138	137	75	40
p	/	0.9403 (a:b)	< 0.0001 (a:c)	/
p	/	/	< 0.0001 (b:c)	/

Note: 1. BV - bacterial vaginosis, VVC - vulvo-vaginal candidiasis.
2. "a" - BV, "b" - intermediate BV, "c" - VVC.
3. "a:b", "a:c", and "b:c" signify the differences between the prevalences of each condition.
4. Unknown pathogenic vaginitis refers to vaginitis with clinical symptoms and increased leukocytes but with no common pathogens identified microscopically.
5. "/" : Items and values were not compared.

Table 3. Incidences of vulvo-vaginal infections by age in girls undergoing rapid puberty.

Age group	n	BV, n (%)	Intermediate type of BV, n (%)	VVC, n (%)	Unknown pathogenic vaginitis, n (%)
9 years old	119	48 (40.34)	52 (42.02)	9 (7.56)	12 (10.08)
10 years old	106	36 (33.96)	40 (37.74)	22 (20.75)	8 (7.55)
11 years old	61	23 (37.70)	17 (27.87)	18 (29.51)	3 (4.92)
12 years old	59	17 (28.81)	14 (23.73)	15 (25.42)	13 (22.03)
13 years old	45	14 (31.11)	16 (35.56)	11 (24.44)	4 (8.89)
Total	390	138 (35.38)	137 (35.13)	75 (19.23)	40 (10.26)
p		0.5600	0.1085	0.0020	0.0196

Note: 1. VVC - vulvo-vaginal candidiasis, BV - bacterial vaginosis.
2. Unknown pathogenic vaginitis refers to vaginitis with clinical symptoms and increased leukocytes but with no common pathogens identified microscopically.

which are lower than those of women of childbearing age (> 18 years old). Additionally, girls at this stage are unlikely to be taking long-term contraceptives, which can reduce systemic immunity, or to overuse vulvar cleansing agents, which can decrease vaginal immunity [28]. Furthermore, girls undergoing rapid puberty are less likely to have reduced body resistance from irregular lifestyles and work pressure than are women of

Table 4. Multiple comparisons of the incidences of BV, intermediate BV and VVC by age in girls undergoing rapid puberty.

Comparison of the age groups	BV	Intermediate type of BV	VVC
	p	p	p
A:B	0.3238	0.5129	0.0042
A:C	0.7324	0.0631	< 0.0001
A:D	0.1328	0.0167	0.0010
A:E	0.2770	0.4515	0.0032
B:C	0.6261	0.1954	0.2019
B:D	0.4972	0.0661	0.4907
B:E	0.7335	0.7997	0.6158
C:D	0.3016	0.6045	0.6164
C:E	0.4815	0.3982	0.5632
D:E	0.7997	0.1872	0.9090

Note: VVC - vulvo-vaginal candidiasis, BV - bacterial vaginosis, A - 9-year-old group, B - 10-year-old group, C - 11-year-old group, D - 12-year-old group, E - 13-year-old group.

childbearing age, which can lead to a greater incidence of VVC than of BV in these girls. Here, the BV incidence was highest in girls undergoing rapid puberty, and we found no significant differences among age groups. BV incidence is related to low estrogen levels as well as poor hygiene habits, indirect contact, and microecological imbalances caused by changes in the vaginal environment [29,30]. Menarche is rare in 9-year-old girls, and a lack of menstrual hygiene in older girls increases their BV incidence [31]. Additionally, the average age of girls in China when entering junior high school for compulsory education is 12 years, meaning that these girls are leaving their parents' care and spending most of their time living in independent group accommodations [31]. Without adequate mental development, full awareness of sexual health, and parental care, these girls are susceptible to poor hygiene and indirect contact leading to BV infections. For these reasons, BV incidence does not significantly differ among age groups.

The VVC incidence was significantly lower in 9-year-old girls than in other age groups. This may have been due to higher estrogen levels and glycogen secretion than in other age groups, which can change the internal vaginal environment, including the pH, and enhance the adherence of *Candida* spp. to cause VVC infections [32,33]. Additionally, the lower VVC incidence may have been due to the increasing stress of studying among girls over 10 years old, which can increase the probability of opportunistic VVC infections from decreased immunity owing to the lack of proper physical exercise, irregular work and life [34]. Moreover, 9-year-old girls have no menarche compared with older girls undergoing rapid puberty, thus providing *Candida* spp. with a warm and humid reproductive environment [31]. Combined with poor hygiene, this can cause VVC infections. Girls over 10 years old experience menarche to some degree, thus increasing the chances of *Candida* spp. infection and reproduction. These factors thus lower the VVC incidence in 9-year-old girls compared with that in older girls. In this study, the positivity rate of *C. albicans* was significantly higher than that of *NAC*, which was consistent with the results of a previous study on women of childbearing age [35]. This study also indicated that in girls undergoing rapid puberty who are infected with VVC, the VVC infection may have been caused by the same factors as in women of childbearing age, and *C. albicans* is more likely to cause opportunistic VVC infections.

Trichomoniasis is a vaginal inflammation caused by exogenous vaginal *Trichomonas vaginalis* through indirect or direct contact with infected individuals [14]. *Trichomonas vaginalis* has strong vitality, can adapt to different environments, and can grow and reproduce at 25°C - 42°C. It can survive for at least 21 days at 3°C - 5°C, for 20 - 60 minutes at 46°C, and for several hours after leaving the human body under semi-dry conditions [36]. Therefore, the main modes of *Trichomonas vaginalis* transmission are direct infection by sexual behavior and indirect infection from public places such as swimming pools [37]. We found no trichomoniasis in the current study. Girls undergoing rapid puberty are young and exhibit immature physical development. Furthermore, their parents often supervise their behavior, thus minimizing the ability to spread trichomoniasis through direct sexual contact with someone infected with *Trichomonas vaginalis* [36]. Additionally, studies have shown that susceptibility to trichomoniasis may be related to the vaginal microbial community and immune-mediated factors [38]. Even if *Trichomonas vagi-*

nalis is present in public places, the special intravaginal environment and protective structure of girls at this stage may prevent potential indirect infections. *Trichomonas* infections differ from vulvo-vaginal infections in girls aged 14–18 years who are undergoing late puberty. Trichomoniasis occurs during late puberty and is mostly caused by direct contact with the *Trichomonas vaginalis* pathogen via sexual behavior [39]. These factors are closely associated with parents providing inadequate gender education and to educational institutions providing inadequate sexual health education for adolescents [40, 41].

Our study had some limitations. First, we used primarily morphological observation and retrospective analysis, which may have led to some limitations in the diagnostic criteria, such as in the diagnosis of VVC. We will use PCR or molecular biological methods to diagnose various types of vaginitis in future studies. Second, because this was a retrospective analysis, there were some limitations in the inclusion and exclusion criteria. The results and conclusions may have been affected by unknown factors. Finally, girls undergoing rapid puberty with elevated leukocytes were initially included in the statistical analysis of vaginitis. However, increased leukocytes in the vaginal discharge could be caused by cervicitis or pelvic inflammation rather than vaginitis; these patients should be excluded from future studies.

CONCLUSION

Girls undergoing rapid puberty can easily develop BV and VVC, and the incidence of BV is significantly higher than that of VVC. The positive rate of *C. albicans* in VVC infections is higher than that of *NAC.* This is related to estrogen levels and the vaginal environment at this developmental stage as well as to personal hygiene, body resistance, and parental care. Therefore, attention should be paid to the impacts of menarche and pubertal development on vulvo-vaginal infections in girls at this stage. Additionally, to reduce the occurrence of vulvo-vaginal infections and improve the physical and mental health of adolescent girls, medical staff should provide better health education for girls undergoing rapid puberty. Girls at this stage should also be more physically active to improve their own immunity and resistance. Everyone should pay attention to public hygiene and help reduce the chance of contact infections. Furthermore, attention should be paid to the occurrence and prevention of BV and VVC in girls undergoing rapid puberty, especially to VVC caused by *C. albicans* infection. If these girls present symptoms of vulvo-vaginal infections, timely medical treatment should be sought. If BV or VVC is confirmed, timely anti-infective treatments should be provided, and the vaginal microecology should be adjusted to prevent recurrence.

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Ethical Approval:
All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent:
Informed consent was obtained from all participants included in the study.

Declaration of Interest:
All authors declare that they have no conflicts of interest.

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