

Ureaplasmas and Mycoplasmas in Vaginal Samples from Prepubertal Girls and the Reasons for Gynecological Consultation

Patricia Romero MD^{1,*}, Mónica Muñoz MD¹, María Angélica Martínez PhD², María Inés Romero MD³, Laura Germain MD⁴, Margarita Maida MD⁴, Viviana Quintanilla⁴, María Teresa del Río⁴

¹ Pediatric Gynecology Unit, Luis Calvo Mackenna Children's Hospital, Santiago, Chile

² Institute of Biomedical Sciences, Universidad de Chile, Santiago, Chile

³ Public Health Department, Pontificia Universidad Católica, Santiago, Chile

⁴ The PREVIF Foundation, Santiago, Chile

ABSTRACT

Objectives: The aim of the study was to evaluate vaginal colonization with *Ureaplasma urealyticum* (UU) and *Mycoplasma hominis* (MH) in prepubertal girls and reason for gynecological consultation.

Patients and Methods: All prepubertal girls sent for consultation for medical issues to a pediatric gynecology department. Vaginal swabs were obtained for culture and were seeded using specific media. Patients colonized with genital mycoplasmas (GMs) were evaluated by a psychologist to rule out sexual abuse (SA).

Results: A total of 119 patients were included. The mean age was 5.9 y. Reasons for consultation were vulvovaginitis in 78 (66%), SA before study entry in 19 (16%), labial adhesion in 8 (7%), genital bleeding in 8 (7%), suspected sexual abuse in 3 (3%) and 1 patient was sent for consultation for labial adhesion but had a normal examination (1%), physical neglect in 1 (1%), and genital ulcers in 1 (1%). UU was isolated in 14 (12%) MH was isolated in 3 (3%). UU was isolated in 9 patients (47%) with SA before study entry. Five patients colonized with UU that had consulted for other reasons were evaluated by a pediatric psychologist; 4 disclosed SA. One patient colonized with UU did not disclose SA. Patients with GMs were more likely to disclose sexual abuse (UU $P < .0001$. MH $P < .0065$).

Conclusion: GMs were isolated more in SA cases. Patients colonized with GMs and consulted for other issues than SA were more likely to disclose SA.

Introduction

Genital mycoplasmas (GM), including *Mycoplasma hominis* (MH) and Ureaplasmas (*Ureaplasma parvum* and *Ureaplasma urealyticum*, UU), are frequently found in the adult genital tract. Genital colonization increases with sexual activity, more quickly in women than in men, which indicates that the female genital tract is more susceptible to colonization by these organisms.¹ Data suggests that 40%–80% of pregnant women are vaginally colonized with GMs.² Genital colonization is significantly higher in sexually active non-pregnant women than in those sexually inactive, therefore prepubertal patients are unlikely to be colonized with GMs.³ Other studies of microbiological isolation from the vagina and cervix in adolescents with sexual activity have shown that UU colonization increases with sexual activity.⁴ People carrying mycoplasmas tend to be asymptomatic so they do not even know they are carriers. GM symptoms are also quite general and infections are often associated with sexually transmitted disease (STDs) in adults.⁵

Vertical transmission usually occurs at birth during passage through the birth canal and less frequently due to an ascending intrauterine infection.⁶ There are a small number of manuscripts that have studied GMs in

prepubertal girls. UU colonization gradually disappears during the first year of life in approximately one-third of newborns exposed.^{6,7} Most frequently colonization sites are in the nasopharyngeal, rectal, and vaginal mucosa. Studies have demonstrated that 42%–53% of newborns exhibit UU colonization in the vagina; however, UU colonization persists for no more than 8 days and up to 3.3 months.^{8,9} Neonatal colonization with MH is rare and persists for only a few days in newborns.⁷ In relation to colonization with GMs in prepubertal girls there are few and old publications that do not always separate the groups according to pubertal stage.^{10–12} There are studies relating hormonal status with colonization with GMs in the female vagina.³ However, recent studies have found a significant correlation between pubertal status and vaginal colonization with UU.⁷

A few years ago we did not routinely take culture samples from pediatric gynecological patients unless specifically requested. We had a patient that developed uveitis and UU was isolated in the vaginal microbiology study. Following this a psychological assessment was done and the patient disclosed sexual abuse by her grandfather. Therefore we realized how important it was to study vaginal GMs in the prepubertal age group and to consider psychosocial evaluations specifically in cases where GMs are isolated. So this experience motivated us to investigate GMs isolation in prepubertal patients consulting for gynecological issues at our gynecology department.

The authors indicate no conflicts of interest.

* Address correspondence to: Patricia Romero, MD, Antonio Varas 360, Providencia, Santiago, Chile; Phone: 56-02-25755963.

E-mail address: patiromerof@yahoo.com (P. Romero).

Patients and Methods

Subjects

This study was conducted at the Pediatric Gynecology Department (PGD) of Luis Calvo Mackenna Public Children's Hospital in Santiago, Chile from August 2008 to October 2009. Criteria selection was defined by Tanner I for both pubic and breast exam. All prepubertal patients attending the PDG for evaluation for a gynecological condition including vulvovaginitis; labial adhesion; genital bleeding; genital ulcer; physical neglect; suspected or had been sexually abused previously were included.

In relation to the cases of positive sexual abuse;

- (1) Patients had disclosed sexual abuse during a psychological evaluation before entering the study.
- (2) Were living under legal protection after sexual abuse.
- (3) Patients had a previous STD compatible with the presence of an infection confirming mucosal contact with infected genital secretions.

Exclusion criteria were:

- (1) Antibiotics for less than 15 days.
- (2) Pubertal patients as defined by Tanner II for pubic and breast exam.

The study was approved by the hospital ethics committee, and informed written consent was obtained from the parents or the patient guardians.

Microbiology

Two trans-hymeneal swabs were used to collect vaginal specimens from each patient. The swabs were placed into a sucrose phosphate buffer (2 SP) Stuart transport medium. The specimens were immediately transported to the microbiology laboratories at the Universidad de Chile. Aliquots of 0.1-ml specimens in 2 SP were inoculated in arginine and urea broth tubes and placed in Agar A7 plates for a GM diagnosis.^{5,6} For the diagnosis of facultative anaerobic bacteria and yeasts, the specimens in the Stuart transport medium were seeded in chocolate and blood agar, Columbia agar, MacConkey and Sabouraud agar. *Trichomonas vaginalis* were studied in wet mounts. *Neisseria gonorrhoeae* was studied in Thayer Martin agar. Herpes virus was identified through Tzanck preparation.¹²

Psychological Evaluation

Patients who were consulted for reasons other than sexual abuse and who were colonized with GMs were evaluated by the PREVIF Foundation, which is an interdisciplinary center that performs psychosocial evaluations in cases of child abuse. An interview was conducted between the patient's caregiver and a social worker, followed by 7 individual patient sessions with a pediatric psychologist. These sessions included semi-structured interviews, non-inductive questioning, and play sessions with anatomical dolls and dolls that represented family members and other

figures. Standard psychological tests were administered, including a testing graphic, the Javiera and Roberto test and the Thematic Apperception Test. Sexual abuse cases were defined as patients who disclosed genital touching and/or genital or anal penetration during an evaluation with a psychologist.

Statistical Analysis

The collected information was entered into a Microsoft Excel spreadsheet and analyzed. Descriptive statistics were used, and the results were expressed in absolute numbers and relative frequencies. Fisher's exact test was applied to determine the significance of the GMs. A *P* value < .05 was considered statistically significant.

Results

A total of 119 patients Tanner I participated in the study, the mean age of the participants was 5.9 years (range = 8 months to 10.5 years). The reasons for consultation were vulvovaginitis in 78 patients (66%), sexual abuse in 19 patients (16%), and labial adhesion in 8 patients (7%), vaginal bleeding in 8 patients (7%), suspected sexual abuse in 3 patients (3%), 1 patient was referred due to labial adhesion but the genital exam was normal. There was physical neglect in 1 patient (1%), and genital ulcers in 1 patient (1%). UU was isolated in 14/119 patients (12%). The patients with UU consulted for the following reasons: 2 patients (3%) for vulvovaginitis, 9 patients (47%) for sexual abuse previous to entering study, 2 patients (67%) for suspected sexual abuse and 1 patient for genital ulcers. MH was isolated in 3/119 (3%) patients, 2 were sexually abused previous to entering study and 1 with genital ulcers was diagnosed with the herpes virus. See [Table 1](#).

Overall, 9/14 patients who were 3–8 years of age had disclosed sexual abuse before entering the study. Of these 9 patients, 7 had been sexually abused by a male relative or family friend. Of the 9 patients, 2 had contracted gonorrhea before entering the study (1 case 2 weeks before and the other 8 months before) and *G. vaginalis* was isolated in 2 other patients during the study ([Table 2](#)).

GM colonization was found in 5/14 patients who consulted for reasons other than sexual abuse, 3/5 patients disclosed sexual abuse during the psychosocial evaluation and 1 patient had genital ulcers with herpes virus diagnosed by Tzanck test and was also infected with *G. vaginalis*

Table 1

Number and Percentage of Cases Colonized with Ureaplasmas and/or Mycoplasma and Reasons for Consultation

Reason for consultation	Cases n (%)	UU cases n (%)	MH cases n (%)
Vulvovaginitis	78 (65)	2 (3)	-
Sexual abuse	19 (16)	9 (47)	2 (11)
Labial adhesion	8 (7)	-	-
Genital bleeding	8 (7)	-	-
Suspected sexual abuse	3 (2)	2 (67)	-
Gynecological evaluation	1 (1)	-	-
Physical neglect	1 (1)	-	-
Genital ulcer	1 (1)	1 (1)	1 (100)
Total	119	14 (12)	3 (2.5)

MH, *Mycoplasma hominis*; UU, Ureaplasmas.

Table 2
Cases that Were Consulted for Sexual Abuse Colonized with Genital Mycoplasmas

Case	Age (y)	Cause for consultation	Aggressor	GMs	Other micro-organism
1	8	Vaginal discharge. SA. 8 months before	Uncle	UU	
2	4.9	Vaginal discharge. SA. Under legal protection	Mother's partner	UU	<i>G. vaginalis</i>
3	5.3	SA, witnessed by a neighbor	Stepfather	UU	
4	5.6	SA, masturbation, vaginal discharge	Uncle	UU	<i>G. vaginalis</i>
5	8	SA, vaginal discharge	Uncle	UU-MH	
6	3	SA, gonorrhoea treated previously	Neighbour with mental disabilities	UU	
7	4.8	SA, 1 year before, vaginal discharge	Brother	UU-MH	
8	3.4	SA, antecedent of gonorrhoea treated previously	Unknown	UU	
9	7.11	SA, masturbation, vaginal discharge	Stepfather	UU	<i>H. influenzae</i>

GM, Genital mycoplasmas; MH, *Mycoplasma hominis*; SA, Sexual abuse; UU, Ureaplasmas.

disclosed sexual abuse in court. One patient who was consulted for sexual behavior did not disclose sexual abuse during the psychological evaluation. See Table 3.

Patients colonized with GMs were more likely to have been sexually abused; UU $P = .0001$ and MH $P = .0065$.

Discussion

GMs are isolated from genital tract of 60%–80% of sexually active woman and in urethra of 7%–63% of asymptomatic men. In adults, colonization rates with GMs are directly related to sexual activity and to the number of sexual partners.¹³ Vertical transmission with GMs is colonized from mothers to their newborns, but this decreases after 3 months and no GMs are isolated after 2 years of age.⁸

Another study found a relation between this hormonal state and genital tract colonization with GM in 82.1% of UU cases in the genital tract of pregnant females > 36 weeks compared with a significantly lower frequency of UU in prepubertal girls (5%), women approximately 30 days puerperium (24%) and postmenopausal women (25%).³ This data supports the fact that GM vaginal colonization is favored by sexual activity and that it exists in hormonal conditions that favor vaginal GMs so this would explain why the isolation of GMs colonization in prepubertal girls is low.

This study was done only in prepubertal girls so the hormonal estrogen status could not have influenced vaginal colonization, based on the hypothesis that the estrogenic environment favors GM growth.³

Other investigations have examined the frequency of vaginal colonization with these microorganisms in girls, but these studied populations were not divided according to pubertal development as determined by Tanner classification.^{10–12} In addition, recent studies have found a significant correlation between pubertal status and vaginal colonization with ureaplasmas.¹⁴ Therefore, GM isolation is uncommonly found in vaginal microbiology

samples studied from prepubertal girls. This study found 14 (12%) of prepubertal female patients with GMs, 9/14 had disclosed previous sexual abuse before entering the study. Also, GMs were isolated in 5/14 patients who were seen for other reasons such as vulvovaginitis (2), genital ulcers (1), compulsive masturbation (2); however, 4 of these patients disclosed sexual abuse during the psychosocial evaluation.

The significance of GMs findings in prepubertal girls is controversial. However this study supports the fact that patients with UU were more likely to disclose sexual abuse. Frequently sexual abuse does not leave physical elements that leave suspicion, and the incidence of the most recognized STDs in sexually abused children is very low (<3%),¹⁵ so it is important to detect GMs. A limitation of this study was the lack of a healthy control population group of prepubertal girls as it was difficult to obtain permission from parents and the approval from the ethics committee to conduct psychological evaluations in all patients. A study by Hammerschlag included a control group, and the findings on vaginal colonization with UU were significant and support our results.¹¹ In a study of vaginal microbiota in abused and non-abused girls, Gardner¹⁶ isolated *Mycoplasma sp* in 4% of abused girls and in none of those who were not abused. (Shafer studied the vaginal microbiota in adolescents comparing sexual activity; UU was isolated in 80% of the sexually active adolescent adolescents and in only 20% of those with no sexual activity. MH was isolated in 45% of the sexually active adolescents and in only 3% of those with no sexual activity.⁴

GM culture is an easy, sensitive, safe and cost-effective method to obtain UU and/or MH. Also our findings support how important it is to carry out a psychosocial evaluation in prepubertal girls with GMs even if sexual abuse has not been disclosed and to increase awareness for suspected sexual abuse in prepubertal girls who are colonized with GMs.

Table 3
Cases Colonized with Genital Mycoplasmas that Were Consulted for Other Causes and Disclosed Sexual Abuse during Psychosocial Evaluation or in Court

Case #	Age (y)	Cause for consultation	Disclosed SA	Aggressor	GMs	Other micro-organism
1	7	Compulsive masturbation, sexual behavior	Yes	Uncle	UU	
2	4	Vulvovaginitis	Yes	Grandfather	UU	
3	7.2	Vulvovaginitis	Yes	Mother's partner	UU	
4	7	Genital ulcers	In court	Father	UU	<i>G. vaginalis</i>
5	8.7	Erotic sexual behavior	No		MH UU	

GM, Genital mycoplasmas; MH, *Mycoplasma hominis*; SA, Sexual abuse; UU, Ureaplasmas.

It is not enough to question only the main caregivers of young children about sexual abuse; it is more reliable to carry out a system of psychological evaluations in different sessions by a trained pediatric psychologist specializing in SA.

The main impact is that the isolation of GMs would contribute to confirming sexual abuse in prepubertal girls who remain silent and begin legal protection in order to protect the children from future psychological trauma.

References

- McCormack WM, Rosner B, Lee YH: Colonization with genital mycoplasmas in women. *Am J Epidemiol* 1973; 97:240
- Pinna GS, Skevakib CL, Kafetzis DA, et al: The significance of *Ureaplasma urealyticum* as a pathogenic agent in the pediatric population. *Curr Opin Infect Dis* 2006; 19:283
- Iwasaka T, Wada T, Kidera Y, et al: Hormonal status and mycoplasma colonization in the female genital tract. *Obstet Gynecol* 1987; 68:263
- Shafer MA, Sweet RL, Ohm-Smith MS, et al: Microbiology of the lower genital tract in postmenarchal adolescent girls: differences by sexual activity, contraception, and presence of nonspecific vaginitis. *J Pediatr* 1985; 107:974
- Schlicht MJ, Lovrich SD, Sartin JS, et al: High prevalence of genital mycoplasmas among sexually active young adults with urethritis or cervicitis symptoms in La Crosse. *Wisconsin. J Clin Microbiol* 2004; 42:4636
- Waites KB, Katz B, Schelonka RL: Mycoplasmas and ureaplasmas as neonatal pathogens. *Clin Microbiol Rev* 2005; 18:757
- Foy HM, Kenny GE, Levinsohn EM, et al: Acquisition of mycoplasmas and T-strains during infancy. *J Infect Dis* 1970; 121:579
- Sánchez JP, Regan JA: Vertical transmission of *Ureaplasma urealyticum* in full term infants. *Pediatr Infect Dis J* 1987; 6:825
- Syrogianopoulos GA, Kapatais-Zoumbos K, Decavalas GO, et al: *Ureaplasma urealyticum* colonization of full term infants; perinatal acquisition and persistence during early infancy. *Pediatr Infect Dis J* 1990; 9:236
- Hammerschlag MR, Alpert S, Rosner I, et al: Microbiology of the vagina in children: normal and potentially pathogenic organism. *Pediatrics* 1978; 62:57
- Hammerschlag MR, Doraiswamy B, Cox P, et al: Colonization of sexually abused children with genital mycoplasmas. *Sex Transm Dis* 1987; 14:23
- Ingram DL, White ST, Lyna P, et al: *Ureaplasma urealyticum* and large colony mycoplasma colonization in female children and its relationship to sexual contact, age, and race. *Child Abuse Negl* 1992; 16:265
- Cassell GH, Waites KB, Watson HL, et al: *Ureaplasma urealyticum* intrauterine infection: role in premarity and disease in newborn. *Clin Microbiol Rev* 1993; 6:69 Taylor-Robinson D: The male reservoir of *Ureaplasma urealyticum*. *Pediatr Infect Dis* 1986; 5:234
- Kohlberger P, Bancher-Todesca D: Bacterial colonization in suspected sexually abused children. *J Pediatr Adolesc Gynecol* 2007; 20:289
- Hammerschlag M, Guillen C: Medical and legal implications of testing for sexually transmitted infections in children. *Clin Microbiol Rev* 2010; 23:493
- Gardner JJ: Comparison of the vaginal flora in sexually abused and nonabused girls. *J Pediatr* 1992; 120:872