Transhymenal cultures for sexually transmissible organisms

Alison M Steele, Camille de San Lazaro

Abstract

Vaginal swabs were examined for sexually transmissible organisms in 238 girls seen over a 36 month period from July 1989 to June 1992 inclusive. Three groups were seen (age range 1.5-16.1 years), 165 in group 1 in whom there had been a disclosure of sexual abuse, 35 in group 2 where there was a strong suspicion of sexual abuse, and 38 with vulvovaginitis who formed group 3. The isolation of recognised sexually transmitted organisms was low; however, of group 1 19% were colonised with *Gardnerella vaginalis* compared with 3% of group 2 and 3% of group 3. These figures show significant differences in the proportion of children in each group isolating *G vaginalis*. *Candida* spp was isolated only from children over the age of 8 years and in five of the six girls with *Candida* spp there was concern about sexual abuse. Vulvovaginitis in young children was not associated with vaginal candidiasis. The relation of findings to age is discussed and recommendations are made for testing procedures.

(Arch Dis Child 1994; 71: 423-427)

In the 1980s, in Newcastle upon Tyne, a paediatric forensic service was established for the evaluation of alleged victims of sexual abuse after an escalating pattern of referral, largely from the Northumbria Police.

Approximately 300 children a year are accepted for assessment. For a selected number of children this includes investigation for sexually transmitted disease from all relevant sites in boys and girls.

It has become clear in our practice that elective arrangement for such investigation is eminently more satisfactory for a number of reasons. Testing requires prompt plating and laboratory standby not readily available to acute referrals or those assessed off site; parents and children are often highly distressed at the initial referral and less prepared for the relaxed cooperation required; testing conducted too early after an assault is liable to miss serious infection.

A paediatric special clinic was therefore established in 1989, in consultation with genitourinary physicians and microbiologists. Elective testing for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, herpes simplex, *Gardnerella vaginalis*, and *Candida* spp was instituted for all referrals, this testing reflecting the spectrum of adult infection in the parallel adult clinic.¹ In

1991-2, children referred for the vulvovaginitis syndrome were similarly tested. The results obtained from children investigated in this way for 36 months from July 1989 to June 1992 inclusive are presented.

Methods

The case notes of 238 girls were traced, examined, and included in the study. This was achieved by looking at clinic lists and medical notes held separately, for reasons of confidentiality, of all children having had a genital examination by anyone in the forensic paediatric team over the three year defined time period. Girls in the study had been referred from a variety of sources for a variety of reasons. Most had seen the consultant forensic paediatrician previously for the evaluation of possible child sexual abuse at the request of police, social services, or other health professionals. This group of children were then referred on to the special clinic if there was a statement of penile contact, hymenal damage was found, or vulvovaginal symptoms were reported. Some of the children had been referred for vulvovaginitis. This is the syndrome of vaginal discharge associated with vulval irritation and erythema. In this group examination was usually deferred to coincide with the infection screening process. Some children were seen by other paediatricians initially and were accepted by the forensic team for an infection screen for sexually transmitted disease, at which time physical findings were noted.

Two of the children whose case notes were traced had no record of a screen for herpes, but were included in the study as they had no history suggestive of genital herpetic lesions. In addition, there were 12 girls who might have been eligible for inclusion, but were not included (5% of 250) because either their case notes could not be found, or the microbiological results or genital examination were not fully documented. For the purpose of this study any consensual activity in addition to abuse was considered irrelevant.

Most swabs were taken at the special clinic, located near the public health laboratories. A few were taken with the child in theatre or in the main child abuse assessment centre. Children were screened by a member of the forensic paediatric team, usually by the consultant forensic paediatrician. The children were examined in the lithotomy position on the examining couch or on a carer's lap. Five fine wire mounted urethral swabs were passed through the hymenal orifice atraumatically to make contact with vaginal mucosa without the

Department of Child Health, University of Newcastle upon Tyne, Lindisfarne Centre, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP
A M Steele
C de San Lazaro

Correspondence to:
Dr Steele.

Accepted 20 July 1994

Table 1 Age category and diagnostic groups of 238 girls in the study

Group	Age (years)		
	0-2	3-8	9+
1	3	45	117
2	0	24	11
3	2	29	7

use of a speculum. They were then immediately placed in appropriate transport media and sent to the public health laboratories which had previously been alerted and were standing by to receive and plate specimens.

Only 10 of the 238 children screened required an anaesthetic to do so and in all but one subject this was because of the presence of genital warts needing biopsy and diathermy, the suspected presence of a foreign body, or a detected congenital anomaly in the hymen needing surgical evaluation.

Vaginal swabs for bacteriological investigations were inoculated on to a variety of media: modified New York City agar for *N gonorrhoeae*, Columbia human blood agar for *G vaginalis*, and Sabouraud agar for *Candida* spp. They were incubated under appropriate atmospheric conditions and any organisms cultured identified by standard laboratory methods. Where clinically appropriate, routine culture was also carried out on MacConkey and on blood agar. The Feinberg and Whittington transport medium was examined by direct microscopy for the presence of *T vaginalis*. Examination for *C trachomatis* was by the techniques of direct immunofluorescence and enzyme linked immunosorbent assay (ELISA). Vaginal swabs for virological investigation were cultured for herpes simplex.

Genital findings were documented in the case notes; the laboratory staff were unaware of what these were. Three diagnostic groups were analysed: group 1, those children having disclosed sexual abuse (165); group 2, those children where there was a strong suspicion of sexual abuse (35); and group 3, those children with vulvovaginitis (38) about which there was no concern regarding sexual abuse. In this study a disclosure was defined as a statement made by a child to a professional about sexual interference by a named perpetrator. The children were also divided into age groups corresponding with the possible effect of oestrogen on the genital tract. These were: 2 years or less where exogenous maternal oestrogen might still be having an effect;

Table 2 Type of isolate found in 46 of the 238 girls in the study

Isolate	No of girls
<i>Gardnerella vaginalis</i> *	34
<i>Candida</i> spp	6
<i>Chlamydia trachomatis</i>	3
Herpes simplex	1
Trichomonas	1
Group A haemolytic streptococcus	2
Other	1

*One child had *G vaginalis* and *Candida* spp, one child had *G vaginalis* and herpes simplex type 1.

3-8 years where oestrogen was at a nadir; and those children of 9 years and older in whom there was the possibility that their genital tracts would be under the effect of endogenous oestrogens.

Hymenal damage was determined by a positive finding in case records. Hymenal damage was deemed to be present if posterior transections, attenuation, or nodular scarring was noted. Congenital notches and small projectile remnants on an otherwise clear free edge were often seen and discounted in this study. The diameter of the hymenal orifice was not considered an objective indicator of damage.

Results

The results of transhymenal cultures of 238 children were obtained over a three year time period. Table 1 shows the groups and age categories within which they fell. It can be seen that most of the disclosed sexual abuse victims were 9 years and over and most of the vulvovaginitis group were aged 3-8 years. By definition the vulvovaginitis group were symptomatic. In addition, 80 (48%) of the disclosed group and 28 (80%) of the suspicious group reported vulvovaginal symptoms on direct questioning. Within the group of children disclosing sexual abuse, 101 (61%) reported adult penis to child genital contact and 21 (13%) adult mouth to child genital contact. The true figures may obviously be higher. One hundred and fifty eight (79%) of the 200 children screened because of disclosed sexual abuse or suspicion of sexual abuse had hymenal damage. The relation between disclosure, vulvovaginal symptoms, and hymenal damage for all children is shown in the figure.

There was a total of 48 isolates from 46 children. Details are shown in tables 2 and 3. Eight children in the study had genital warts and of these two had *G vaginalis*. *G vaginalis* was by far the most common organism cultured, 34 (14%) of 238 of the children screened were colonised (mean age 13.4 years). Thirty two (19%) of 165 of the

Relation between disclosure, vulvovaginal symptoms, and hymenal damage of 238 girls in the study.

Table 3 Isolates found within each diagnostic group

Isolate	Group		
	1	2	3
+	37	5	4
-	128	30	34

Table 4 Number of girls with *Gardnerella vaginalis* in each group ($p=0.0019$ by Fisher's exact test)

<i>Gardnerella vaginalis</i>	Group		
	1	2	3
+	32	1	1
-	133	34	37

Table 5 Number of girls with *Gardnerella vaginalis* isolated in each age group

<i>Gardnerella vaginalis</i>	Age (years)		
	0-2	3-8	9+
+	1	1	32
-	4	97	103

disclosed group were positive for *G vaginalis*, whereas only one (3%) of 35 of the suspicious group and one (3%) of 38 of the vulvovaginitis group were positive (table 4). The distribution of *G vaginalis* is significant ($p=0.0019$) using the 3×2 analogue of Fisher's exact test.² The ages of the groups are very different, however (table 5). Of those 34 children isolating *G vaginalis*, 29 (85%) had hymenal damage and a disclosure, one had hymenal damage without a disclosure, and three had no hymenal damage but full disclosures of penile contact. One child had no hymenal damage and no disclosure, was 2.5 years of age, and in the vulvovaginitis group.

Candida was isolated from six children. They were all 9 years or older (mean age 13.8 years). In three there were disclosures of sexual abuse and hymenal damage, in two there was suspicion of sexual abuse with hymenal damage, and in only one child was there no suspicion and no hymenal damage. In one of the children with disclosed abuse and hymenal damage *G vaginalis* was also isolated and in one of the children in whom there was suspicion of sexual abuse and hymenal damage there was concomitant lichen sclerosis et atrophicus. Table 6 gives details of children with other isolates. Organisms were isolated in 42 out of 200 (21%) of the children in the

sexual abuse groups (disclosed or suspicious) and in four out of 38 (11%) of the vulvovaginitis group. The organisms isolated in the latter group were *G vaginalis* (one), *Candida* spp (one), and group A haemolytic streptococcus (two).

Discussion

Infections known to be sexually transmitted in adults, such as gonorrhoea and trichomonas, are usually transmitted this way in children.³⁻⁵ The exception to this is the vertical transmission of organisms from mother to child in utero or during birth, where the time of presentation may vary depending on the organism, severity of infection, and host factors. Although most organisms transmitted from mother to child present in the neonatal period, it is acknowledged that genital warts as a result of human papilloma virus infections may present later.^{6,7}

There remains controversy over the nature of transmission of other organisms, notably *G vaginalis*⁸⁻¹³ and *Mycoplasma* spp.^{14,15} The role of *Candida* spp as a sexually transmitted organism in children has been little discussed, although in adult women there is evidence to suggest that in some instances sexual transmission may be a feature.¹⁶

In our study the rate of isolation of those organisms traditionally regarded as sexually transmitted such as chlamydia, herpes and trichomonas was low, but exclusively within the group of children disclosing sexual abuse. These are important infections to identify and treat.

By far the most prevalent organism was *G vaginalis*, with 32 (19%) of children within the disclosed sexual abuse group having it isolated within their genital tracts. *Gardnerella* was found most often in the group of children disclosing sexual abuse; however, the groups are not strictly comparable because they are of different age distributions. Without a control group it could be argued that the presence of *G vaginalis* in the sexually abused group reflected the different vaginal environment in the group in which it was mostly isolated – that is, over 8 years old. In theory, however, the non-oestrogenised alkaline environment of the prepubertal vagina should provide more favourable conditions for this particular organism.⁸ In the group of children in whom *G vaginalis* was found, there was only one child without either hymenal damage or a disclosure of penile contact and this child was 2.5 years of age.

Previous work on the significance of *G vaginalis* in relation to sexual contact is inconclusive. The two most recent case-control studies reach different conclusions. Gardner describes the outcome of swabs taken from sexually abused girls compared with a control group of girls.¹⁷ The swabs from the latter were taken under anaesthetic for minor elective surgery. *G vaginalis* was found in 15 of 209 cases compared with one of 108 controls, a statistically significant difference. Ingram *et al* compared swabs taken from three groups,

Table 6 Details of girls with isolates other than *Gardnerella vaginalis*

Age (years)	Organism	Abuse history	Perpetrator	Findings
4	Chlamydia, +ve ELISA	Oral intercourse 'tickling'	Stepfather	Fourchette tear, urethral dilatation
6	Group A haemolytic streptococcus	No abuse, history of bloody vaginal discharge	-	Normal
7	Group A haemolytic streptococcus	No history of abuse, bleeding in pants/dysuria	-	Lichen sclerosis et atrophicus, no trauma
12	Trichomonas	Sexualised behaviour, vaginal intercourse	Youth leader	Hymenal tears
12	Faecal organisms	Disclosed sexual assault	Not documented	Hymenal tear, genital warts
14	Chlamydia +ve ELISA -ve DFT	Previous neglect, in care, vaginal intercourse	Multiple	Hymenal tears
14	Chlamydia +ve ELISA +ve DFT	Vaginal intercourse	Father	Gross hymenal damage
15	Herpes simplex type 1*	Extensive exposure to abusing environment, no disclosure	Unknown	Gross hymenal damage

+ve=Positive; -ve=negative **Gardnerella vaginalis* also present.
DFT=direct immunofluorescent technique.

namely 191 girls having disclosed sexual contact or having *N gonorrhoeae* or *C trachomatis*, 144 girls evaluated for possible sexual abuse, and 31 controls (children of friends of the author) and found similar rates of isolation in the three groups.¹⁸ Neither study states exactly where the swabs were taken from, and each related to different populations of children.

Candida is often thought to be an important cause of vulvovaginitis.¹⁹⁻²¹ In our series, surprisingly, *Candida* spp was isolated only in children over 8 years of age. Five of the six children in which vaginal candida was isolated had either alleged sexual abuse or presented signs or symptoms raising the possibility of abuse.

In the vulvovaginitis group two children had group A *haemolytic streptococcus* and were aged 6.25 and 7.75 years respectively; both reported a bloody discharge, which was atypical of the vulvovaginitis group as a whole. One child aged 2.5 years had *G vaginalis* as previously discussed and one child was 11.9 years of age and had *Candida* spp isolated.

We believe that paediatricians involved in the assessment of sexually abused children or children at risk should not lose sight of all aspects of health care and that investigation for sexually transmissible organisms should be part of a comprehensive medical service to these children. We found ourselves unable to predict the presence of significant organisms from history alone and our study suggests that symptomatology relates poorly with the presence of isolate.

Transhymental swabs are recommended for screening for sexually transmissible infection. The process is well tolerated and many children and parents find it reassuring and, indeed, therapeutic (namely, a sense of being 'cleansed'). The children should be properly informed and time taken to position correctly and await full relaxation. With appropriate distraction, children do not resist the procedure, nor do they report any pain. Behavioural symptoms after these sessions have not been reported to us, though we believe that further investigation of the impact of genital examination and allied procedures could be valuable. In our group of 238 children only one child refused to be screened, her symptoms being sufficient to mandate examination under anaesthetic.

Findings of infections such as gonorrhoea or trichomonas may assist in the forensic process; however, of paramount concern must be the implications for the health of children. Such organisms need vigorous treatment and further investigation. The need for treatment of asymptomatic colonisation with *G vaginalis* or *Candida* spp is uncertain; our practice is to treat as for bacterial vaginosis or vaginal candidiasis in adults. Retesting is limited to those children presenting with further symptoms.

In our experience, it is relatively uncommon for older girls to present with symptoms of vulvovaginitis. This relates, presumably, to the protective and lubricating effects of increasing oestrogen output. In practice, it is

therefore possible to reassure parents of younger children that symptoms will resolve in time. Occasionally, symptoms of vulvitis are persistent or severe enough for topical oestrogen applications to be deemed necessary to assist healing. The smaller number in our older age group is to be expected and, indeed, no child presented with vulvitis over the age of 11 years. Pierce and Hart²² reported a study of vulvovaginitis in 200 girls presenting over a period of 33 months to the Alder Hey accident and emergency department. Of these, only 14 were in the 10-15 year age group. *G vaginalis* was not found in any of these 200 children, and candida was found in only seven children, of which six were at the extreme ends of the age range and the seventh in a child with flexural genital psoriasis. In this study swabs were taken from the introitus and vestibule and specific sampling from vaginal mucosa was not sought, perhaps accounting for the greater detection of apparently opportunistic colonisation (*Escherichia coli*, *Haemophilus influenzae*, and streptococcus).

Our study suggests that the presence of *G vaginalis* and *Candida* spp in vaginal cultures relates to a history or physical signs of sexual trauma. There will obviously be ethical and logistical difficulties in testing these findings further with asymptomatic controls. We suggest, however, that clinicians should view the presence of these organisms with strong suspicion, especially in children where hymenal trauma has been found.

We thank Professor Liam Donaldson of the Northern Regional Health Authority for supporting the work of the centre, Dr John Matthews for statistical advice, the public health laboratories of Newcastle General Hospital for advice on microbiological matters, and last, but by no means least, Mrs Julia Temple for secretarial support.

- 1 Newcastle Health Authority. *Annual report on sexually transmitted diseases*. Newcastle upon Tyne: Newcastle Health Authority, 1992.
- 2 Mehta CR, Patel NR. Algorithm 643 Fexact: a FORTRAN subroutine for Fisher's exact test on unordered rxc contingency tables. *ACM Transactions on Mathematical Software* 1986; 12: 154-61.
- 3 Neinstein LS, Goldenring J, Carpenter S. Non sexual transmission of sexually transmitted diseases: an infrequent occurrence. *Pediatrics* 1984; 74: 67-76.
- 4 Stewart D. Sexually transmitted diseases. In: Heger A, Emans SJ, eds. *Evaluation of the sexually abused child*. Oxford: Oxford University Press, 1992: 145-69.
- 5 Giardino AP, Finkel MA, Giardino ER, Seidi T, Ludwig S, eds. Sexually transmitted disease. *A practical guide to the evaluation of sexual abuse in prepubertal children*. London: Sage, 1992: 100-12.
- 6 Sedlacek TV, Lindheim S, Eder C, et al. Mechanism for human papillomavirus transmission at birth. *Am J Obstet Gynecol* 1989; 161: 55-9.
- 7 Gutman LT, Herman-Giddens ME, Phelps WC. Transmission of human genital papillomavirus disease: comparison of data from adults and children. *Pediatrics* 1993; 91: 31-8.
- 8 Muram D, Buxton BH. Gardnerella vaginalis in children: an indicator sexual abuse. *Pediatric and Adolescent Gynecology* 1984; 2: 197-200.
- 9 Hammerschlag MR, Cummings M, Doraiswamy B, Cox P, McCormack WM. Non specific vaginitis following sexual abuse in children. *Pediatrics* 1985; 75: 1028-31.
- 10 Bump RC, Sachs LA, Buesching III WJ. Sexually transmissible infectious agents in sexually active and virginal asymptomatic adolescent girls. *Pediatrics* 1986; 77: 488-94.
- 11 Bartley DL, Morgan L, Rimsza ME. Gardnerella vaginalis in prepubertal girls. *Am J Dis Child* 1987; 141: 1014-7.
- 12 De Jong AR. Vaginitis due to Gardnerella vaginalis and to Candida albicans in sexual abuse. *Child Abuse Negl* 1985; 9: 27-9.
- 13 Bump RC, Buesching III WJ. Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exclusive sexual transmission. *Am J Obstet Gynecol* 1988; 158: 935-9.

- 14 McCormack WM, Almeida PC, Bailey PE, Grady EM, Lee Y-H. Sexual activity and vaginal colonisation with genital mycoplasmas. *JAMA* 1972; 221: 1375-7.
- 15 Hammerschlag MR, Doraiswamy B, Cox P, Cummings M, McCormack WM. Colonisation of sexually abused children with genital mycoplasmas. *Sex Transm Dis* 1987; 14: 23-5.
- 16 Horowitz BJ, Edelstein SW, Lippman L. Sexual transmission of candida. *Obstet Gynecol* 1987; 69: 883-6.
- 17 Gardner JJ. Comparison of the vaginal flora in sexually abused and non abused girls. *J Pediatr* 1992; 120: 872-7.
- 18 Ingram DL, White ST, Lyna PR, et al. Gardnerella vaginalis infection and sexual contact in female children. *Child Abuse Negl* 1992; 16: 847-53.
- 19 Paradise JE, Campos JM, Friedman HM, Frishmuth G. Vulvovaginitis in premenarcheal girls: clinical features and diagnostic evaluation. *Pediatrics* 1982; 70: 193-8.
- 20 Altchek A. Pediatric vulvovaginitis. *J Reprod Med* 1984; 29: 359-75.
- 21 Sweet RL, Gibbs RS. Infectious vulvovaginitis. *Infectious diseases of the female genital tract*. 2nd Ed. Baltimore: Williams and Wilkins, 1990: 216-28.
- 22 Pierce AM, Hart CA. Vulvovaginitis: causes and management. *Arch Dis Child* 1992; 67: 509-12.

The importance of clonality – Langerhans' cell histiocytosis

In the latest edition of the leading British textbook of paediatrics it is stated that the group of diseases we all used to know as histiocytosis X 'are not malignancies but proliferations probably secondary to defects of immunoregulation'. Work in several centres in the United States (Cheryl L Willman and colleagues, *New England Journal of Medicine* 1994; 331: 154-60) now casts doubt on that thesis.

As you know, in females each somatic cell randomly inactivates either the maternal or the paternal X chromosome early in embryonic development (lyonisation). After that all progeny cells have the same X inactivation as their progenitor cell. Tissues derived by polyclonal expansion will therefore have mixed X inactivation but those derived monoclally will contain cells all with the same inactivated X chromosome. Monoclonal proliferation is taken to be neoplasia.

These workers used X linked polymorphic DNA probes to examine tissues from 10 female patients aged 3 months to 25 years with Langerhans' cell histiocytosis (LCH). Three had acute disseminated LCH (Letterer-Siwe disease), four had the intermediate form (Hand-Schüller-Christian syndrome), and three had a single bony lesion (eosinophilic granuloma). The patterns of X chromosome inactivation in biopsy material were compared with those in blood. Using a probe for the X linked human androgen receptor gene proved informative in all 10 patients and clonal cells were demonstrated in the tissue lesions of nine. In one patient clonality could not be assessed because of constitutional asymmetrical lyonisation. The estimated proportion of clonal cells in the LCH lesions varied from 20 to 65%. There was a high correlation between the percentage of clonal cells detected and the percentage of CD1a – positive histiocytes in a lesion suggesting that the two are the same.

These results indicate that all the clinical forms of LCH are clonal neoplastic disorders. The authors hypothesise that the different clinical forms may arise from different mutations in somatic cells and that, although the demonstration of clonality alone does not give any indication as regards prognosis, it might be possible in future to predict the likely clinical course by identifying the specific mutation. This then could be a guide to appropriate treatment.

ARCHIVIST