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Research Article

Screening for Sexually Transmitted Infections in Adolescents with Genitourinary Complaints: Is There a Still Role for Endocervical Gram Stains?

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Abstract

Study objective: Adolescent females are disproportionately affected by Sexually Transmitted Infections (STIs). Endocervical Gram stain smears taken during gynecological examination are inexpensive, relatively easy procedures to perform and interpret. The purpose of this study was to evaluate the performance characteristics of Gram smears in the diagnosis of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC) and *Trichomonas vaginalis* (TV) in a female adolescent population presenting to the Emergency Department (ED) with genitourinary complaints.

Methods: This study was a retrospective, cohort analysis of consecutive females (ages 13 – 19) seen at three academic medical centers over a two-year study period. All patients underwent a pelvic exam with diagnostic testing for STIs. Positive criteria for a Gram stain included greater than ten white blood cells per high-power field, gram-negative intracellular/extracellular diplococci (suggesting GC), or direct visualization of TV organisms. Polymerase Chain Reaction (PCR) assays were used as the gold standard definition for CT/GC infection. Direct microscopic visualization of organisms on a separate wet mount prep was considered the gold standard for TV infection. Demographic information, clinical findings of cervicitis, and the results of diagnostic testing were obtained from ED records using standardized abstraction forms.

Results: During the study period, 1303 adolescent females were evaluated for genitourinary complaints. A total of 181 adolescents (12.9%) had at least one documented STI. Overall, 298 patients (22.9%) had positive gram stains. The sensitivity, specificity, and positive likelihood ratio for Gram stain in the diagnosis of STI were 28.7% (95% CI, 22.2 to 35.9), 78.1% (95% CI, 75.5 to 80.5), and 1.31 (95% CI 1.0 to 1.7), respectively. The sensitivity of Gram stain to *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Trichomonas vaginalis* were 35.9% (95% CI 26.8 to 45.8), 34.5% (95% CI 18.0 to 54.3), and 5.7% (95% CI 1.3 to 15.7), respectively. The consistency of the data recording was excellent, with a median kappa statistic of 0.89.

Conclusions: The positive likelihood ratio of inflammation on endocervical Gram stain is too low to recommend its use to direct empiric treatment of adolescents at risk for sexually transmitted infections. Diagnostic uncertainty or treatment failures should prompt specific laboratory testing.

Introduction

Adolescents are disproportionately affected by Sexually Transmitted Infections (STIs) including *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* (TV). Of approximately 20 million new STIs annually in the United States, half occur among adolescents aged 15–24 years [1]. It is estimated that one in four sexually active adolescent females has an STI [2]. STIs can lead to serious health issues, especially when untreated. For example, infections like chlamydia and gonorrhea can cause Pelvic Inflammatory Disease (PID), which may result in infertility or chronic pelvic pain [3]. Adolescents are also at higher risk of co-infections with multiple STIs, further complicating health outcomes [4]. Despite the substantial impact of STIs on adolescents, most studies and clinical guidelines have historically focused on

adult populations [2]. This is problematic because adolescents have unique needs in terms of prevention, diagnosis, and treatment. For instance, this age group often faces challenges in seeking healthcare due to concerns about confidentiality, cost, and stigma.

Adolescents account for an estimated 15 million Emergency Department (ED) visits annually [4]. EDs often act as a primary point of care for many individuals who either lack a primary care physician or are unable to access other health resources for screening, education, or treatment of STIs. This setting is not optimal as suggested by several studies showing both over-treatment and under-treatment of chlamydia and gonorrhea as well as increased costs for testing these diseases in emergency care facilities [2-5]. The reasons for over and under-treatment issues in the ED setting include vague empiric treatment guidelines coupled with increased time from specimen testing to getting test results and inadequate follow-up [6]. With a turnaround time of at least 1 to 2 days, laboratory-confirmed results of traditional *N gonorrhoeae* and *C trachomatis* nucleic acid amplification tests are rarely available during a patient's ED visit. Consequently, treatment decisions hinge on the emergency physician's clinical judgement. Undertreatment of patients with a confirmed sexually transmitted infection increases the risk of transmission to subsequent sexual partners and potentially serious infectious complications. Overtreatment of women without a confirmed sexually transmitted infection results in excessive and inappropriate antibiotic use, a key driver of emerging antibiotic resistance [7,8].

A Gram stain is a rapid and inexpensive test available in many Emergency Departments (EDs). It has historically been a critical tool in diagnosing STIs, particularly gonorrhea, as it allows for rapid identification of the causative bacteria, which appear as gram-negative diplococci under a microscope [3]. In women with positive cervical culture results, the Gram stain results from the endocervix are 50% - 60% sensitive and 82-97% specific. In addition, the presence of more than 10 PMNs per high-power field on an endocervical smear is consistent with cervicitis [9]. While Gram stain results alone are insufficient for the diagnosis of STIs in women, the results may be used in combination with clinical symptoms. In many EDs, the gram-stain evaluation of the endocervical flora may provide the only immediate laboratory evidence on which a clinical diagnosis can be based [10]. For these reasons, a clear understanding of the diagnostic metrics of this test in a pragmatic setting is needed. Our aim was to evaluate the performance characteristics of endocervical Gram smears in the diagnosis of *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis* in a female adolescent population presenting to the ED with genitourinary complaints.

Methods

We conducted a retrospective, cohort analysis of consecutive females (ages 13-19) with lower abdominal pain or genitourinary complaints seen at three academic medical centers over a two-year study period. These hospitals included Corewell Health Hospital-Butterworth Campus, Corewell Health Hospital-Blodgett campus, and DeVos Children's Hospital. The combined annual ED census was approximately 190,000 during the study period. The common characteristic of these ED patients was that their chief complaint led the emergency physician to perform a pelvic exam with diagnostic testing for STI using endocervical/vaginal swabs. Positive criteria for a Gram stain included greater than 10 white blood cells per high-power field, gram-negative intracellular/extracellular diplococci (suggesting GC), or direct visualization of TV organisms. Quantitative Polymerase Chain Reaction (PCR) assays were used as the gold standard definition for CT/GC infection. The specimens were processed by using PCR amplification assays for both GC and CT (Abbott m2000, Des Plaines, IL). The turnaround time for study results was approximately 24 h among participating hospitals. Direct microscopic visualization of organisms on a separate wet mount prep was considered the reference standard for TV infection. Adolescents who had repeat visits to the ED for the same STI, which was initially untreated or undertreated, were excluded from the study. Additional exclusion criteria included patients with missing data, or those who presented after sexual assault since each hospital has different guidelines for empiric treatment for this subgroup of patients.

In patients with positive PCR assays for GC and/or CT, the ED physician or quality assurance coordinator confirmed by chart review whether the patient received appropriate antibiotic therapy according to standard CDC guidelines [10]. The clinician-initiated telephone follow-up for antibiotic treatment in cases with positive results in which the patient was not treated at the time of the ED visit. Each participating hospital kept a log of the medical record including test results, how long it took to treat each positive patient, and a record of all patients who could not be treated. Successful follow-up was defined as contacting the patient and providing proper antibiotics. The number of attempts and the reasons for unsuccessful follow-up were also noted.

Standardized abstraction forms were used to obtain relevant patient information from ED medical records (**Appendix**). Patient demographics included age, race/ethnicity, insurance status, primary care physician, pregnancy, and risk factors for STI. Clinical features included signs, symptoms, duration, and pain intensity. Using a 0 to 10 numeric rating scale, the triage nurse asked patients to rate their pain intensity. Laboratory data provided urinary analysis, pregnancy tests, Gram stain results, wet mount, and PCR results for GC or CT. Treatment

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administered in the ED and prescriptions given to the patient on telephone follow-up were subsequently recorded.

All data were collected by four research associates who were blinded to the study objective. Research staff were trained in data abstraction using a set of mock case records. One researcher oversaw data collection and confirmed that variable definitions were uniformly applied. A second investigator performed a blinded critical review of a random sample of 10% of the programs to determine the reliability of data collection using kappa statistics. The primary outcome of this study was to evaluate the performance characteristics of endocervical Gram smears in the diagnosis of STI using sensitivity, specificity, and Likelihood Ratio (LR). We used 95% Confidence Intervals (CIs) for quantifying uncertainty. The PCR results were used as the gold standard to calculate sensitivity, specificity, and likelihood ratios of endocervical Gram stains for CT/GC infection and the wet mount was used as the reference standard for TV infection. We used 95% confidence intervals (CIs) for quantifying uncertainty. Secondary outcomes were the proportion of adolescent females being untreated in the participating EDs, the time to treatment, and the proportion lost to follow-up. Patients were considered lost to follow-up after three telephone attempts to establish communication and a written notice was sent to their provided address.

Data were entered into the Microsoft Excel database (Microsoft Corporation, Redmond, WA). Analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL). Descriptive statistics were used to describe demographic variables and clinical findings. Results were expressed as the mean ± SD. Discrete variables were analyzed by two-tailed unpaired *t* - tests, and Wilcoxon rank-sum and Chi-squared tests for continuous and ordinal data. This retrospective study was approved by the Corewell Health Institutional Review Board (# 2021-164).

Results

During the study period, 1303 adolescent females were evaluated for genitourinary complaints. The mean age was 17.4 + 1.8 years. Common symptoms included abdominal/pelvic pain (53.0%), nausea/vomiting (44.4%), abnormal vaginal discharge (37.2%), urinary complaints (29.3%), and vaginal bleeding (6.6%). The average duration of symptoms prior to presentation was 5.3 + 6.0 days. A total of 318 adolescents (24.4%) had a positive pregnancy test documented in the ED.

Overall, 181 adolescents (12.9%; 95% CI, 12.5 to 16.3%) had at least one documented STI while 24 patients (1.9%; 95% CI, 1.3 to 2.8%) had two or more infections. The most common STI diagnosis was *Chlamydia trachomatis* documented in 105 adolescents (8.1%). This was followed by *Trichomonas vaginalis* (4.6%) and *Neisseria gonorrhoeae* (3.2%). Table 1 compares the demographics and clinical findings in adolescents with and those without documented STI.

Patients with STIs tended to be younger, had complaints of vaginal discharge/bleeding, pelvic pain, and were more likely to have a positive Gram stain (28.7% *vs.* 21.9%).

A total of 298 patients (22.9%) had a positive gram stain. The sensitivity, specificity, and positive likelihood ratio for Gram stain in the diagnosis of STI were 28.7% (95% CI, 22.2 to 35.9), 78.1% (95% CI, 75.5 to 80.5) and 1.31 (95% CI, 1.0 to 1.7), respectively (Table 2). The sensitivity of Gram stain to *Chlamydia trachomatis, Neisseria gonorrhoeae*,

Table 1: Demographics and presenting complaints in adolescent women with and those without documented sexually transmitted infection (STI).

	Adolescents With STI	Adolescents Without STI	*p* - values
Total#	181 (12.9%)	1122 (86.1%)	
Age (mean ± SD)	17.9 ± 2.0	17.3 ± 1.7 yrs	< 0.001
Ethnicity (% white)	46 (25.4%)	269 (24.0%)	0.166
History of STI exposure	14 (7.7%)	59 (5.3%)	0.228
IUD present	8 (4.4%)	33 (2.9%)	0.281
Previous history of STI	43 (23.8%)	203 (18.1%)	0.092
Duration of symptoms (mean ± SD)	5.6 ± 6.3 days	5.1 ± 6.0 days	0.216
Pain intensity (mean ± SD)	6.1 ± 3.2	6.4 ± 3.0	0.302
Presenting Symptoms (%)			
Vaginal discharge	101 (55.8%)	384 (34.2%)	< 0.001
Nausea/vomiting	91 (50.3%)	487 (43.4%)	0.083
Abdominal pain	84 (46.4%)	519 (46.3%)	0.98
Urinary symptoms	45 (24.9%)	337 (30.0%)	0.162
Pelvic pain	36 (19.9%)	123 (11.0%)	< 0.001
Vaginal bleeding	25 (13.8%)	61 (22.8%)	0.006
Other*	31 (17.1%)	135 (12.0%)	0.056
Positive Gram stain (%)†	52 (28.7%)	246 (21.9%)	0.043
Pregnant (%)	17 (9.4%)	301 (26.8%)	< 0.001

*Other symptoms include fever/chills, malaise, dyspnea, dyspareunia, night sweats, and flank pain.
†A positive Gram stain showed greater than 10 white blood cells per high-power field, gram-negative intracellular/extracellular diplococci (suggesting GC), or direct visualization of TV organisms.

Table 2: Diagnostic indices for endocervical Gram smears in the diagnosis of STIs in a female adolescent population with genitourinary complaints.

Statistic	Value	95% CI
Sensitivity	28.73%	22.26% to 35.91%
Specificity	78.07%	75.54% to 80.46%
Positive Likelihood Ratio	1.31	1.02 to 1.69
Negative Likelihood Ratio	0.91	0.83 to 1.01
Disease prevalence*	13.89%	12.06% to 15.89%
Positive Predictive Value*	17.45%	14.08% to 21.43%
Negative Predictive Value*	87.16%	86.03% to 88.22%
Accuracy*	71.22%	68.68% to 73.67%

* These values are dependent on disease prevalence.

and *Trichomonas vaginalis* were 35.9% (95% CI, 26.8 to 45.8), 34.5% (95% CI, 18.0 to 54.3), and 5.7% (95% CI, 1.3 to 15.7), respectively. Despite these poor diagnostic metrics, the 298 adolescents with a positive Gram stain were statistically more likely to receive antibiotics to empirically treat CT/GC infection ($p = 0.041$).

Of the 1122 adolescents without a documented STI, 329 (29.3%) were treated empirically in the ED with antibiotics for presumed STI. In comparison, of the 145 adolescents who had positive PCR studies for Chlamydia trachomatis and/or Neisseria gonorrhoeae, 72 (49.7%) were not treated with antibiotics in the ED. Despite being combined with other clinical findings, the Gram stain likely contributed to the large numbers of adolescents overtreated (29.3%) or undertreated (49.7%) for CT/GC infection in the ED. Other factors such as age, insurance status, ethnic or cultural background, or known STI exposure were not associated with antibiotic treatment in the ED. Eighteen percent of the untreated STI patients had no complaints of pain or tenderness on pelvic examination. Untreated patients were more likely to have a discharge diagnosis of urinary tract infection (33.3%), vaginitis (30.6%), and first-trimester pregnancy (27.8%).

Of the 72 untreated patients with documented STI, 49 (68.1%) were subsequently contacted by phone and/or mail for antibiotics. The mean time interval between ED presentation and antibiotic treatment in these adolescents was 4.1 days (range, 1- 23 days). Overall, 23 (31.9%) of the untreated adolescents with STI were lost to follow-up. The consistency of data recording between investigators was excellent, with a median kappa statistic of 0.89.

Discussion

Endocervical Gram stains taken during a pelvic examination are easy to perform and interpret. However, the positive likelihood ratio of inflammation on endocervical Gram stain (1.3 in adolescent women) is too low to recommend its use to direct empiric treatment in adolescents at risk for sexually transmitted infections. Diagnostic uncertainty or treatment failures should prompt specific laboratory testing. Our findings align with previously reported studies in adults that suggest that Gram stains should not be used alone as a diagnostic method for CT and NG [11-14]. However, this test continues to be used in many settings, including those that provide dedicated STI testing [3,4,15]. The children's hospital affiliated with this study performs over 3000 endocervical gram stains each year.

Adolescent patients with STIs often present to the ED with vague symptoms that lead to an unclear diagnosis [3,4]. PCR assays for GC and CT may return several days after the initial visit, and studies have shown significant loss-to-follow-up rates for untreated adolescents [16,17]. A previous study at our institution showed that approximately 70% of adolescents aged 13–19 years with a positive PCR test result for GC or CT were not initially treated in the ED [18]. Furthermore, over 40% of those patients discharged from the ED without treatment were unable to be reached by telephone or mail, and thus were "lost to follow-up" [18]. Due to these difficulties, emergency physicians may choose to empirically treat for STI when they have a positive endocervical gram stain.

Gram stain is a rapid and inexpensive test available in many emergency departments [9]. This is very useful if the clinician has easy access to a microscope because the diagnosis may be made without waiting for PCR results. An excess of leukocytes (more than 10 PMNs per high-power field) in the endocervical mucous is consistent with chlamydial and gonococcal cervicitis [12]. In women who lack a cervix because of hysterectomy, a urethral culture is used to make the diagnosis [9]. Unfortunately, the results of this current study show the sensitivity of gram stain results to be 29% sensitive and 78% specific for cervicitis in women.

Several factors can complicate the diagnosis and empiric treatment of STIs, including pregnancy, atypical presentation, concurrent urine infection, vaginal bleeding, vulvovaginitis, and the age of the patient [10]. Pregnant patients with GC or CT are rarely treated in the ED upon presentation due to a lack of rapid testing and nonspecific complaints [18,19]. Perhaps the main reason for the lack of antibiotic treatment in this specific population is the young age of the patient. However, the age at which young people begin having sexual intercourse has markedly decreased in the United States, and clinicians should consider cervicitis in their differential diagnosis when treating patients even as young as 13 years of age [16-18]. Furthermore, there are ethical-legal questions regarding reporting to proper authorities when STIs are diagnosed in this underage population.

Screening and treating adolescent ED patients for STIs is paramount. If left untreated, STIs may result in further complications, including acute pelvic inflammatory disease, ectopic pregnancy, and infertility [18]. Culturing organisms takes several days and requires close patient follow-up. Rapid point-of-care testing methods, such as DNA probes, have high sensitivity and specificity [9,15]. Ideally, STI testing should be completed in a timely manner, produce accurate results, and be priced affordably [20]. Only then can we expect the rates of pelvic inflammatory disease and other STI morbidities to be reduced in our adolescent population.

Several limitations were present in our study. Given that this was a retrospective review of medical charts, there is inherent variability in the assessment and documentation of clinical information by different providers. Therefore, documentation of outcome variables of interest may not

have been consistent. Another limitation is that some of the patient population findings in our cohort may be more specific to our region and local culture, which may not translate to every adolescent population. Finally, the small number of adolescents with STIs in our population limited the precision of our findings and resulted in wider confidence intervals.

Conclusion

Sexually transmitted infections in adolescents are a significant public health burden that can lead to severe morbidity if left untreated. Emergency departments are often the first and only point of contact for these patients in the healthcare setting. The positive likelihood ratio of inflammation on endocervical Gram stain is too low to recommend its use to direct empiric treatment in adolescents at risk for sexually transmitted infections. Further research should include liberal policies to treat less symptomatic patients empirically, accentuate point-of-contact testing, and better mechanisms for contact and follow-up after hospital or clinic discharge.

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