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Four and a Half Year History of APTIMA® GC/CT Nucleic Acid Amplification Testing (NAAT) in Children Seen for Sexual Abuse

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Lifespan

Learning Objectives: By the end of the presentation, participants should be able to identify the benefits of using NAAT and confirmatory probe testing with APTIMA in child abuse assessment for the STIs, GC and CT.

ABSTRACT (Revised February 2010)

Background: This study describes the 4 year history of STI evaluation with NAAT, specifically the APTIMA® GC/CT assays (Gen-Probe, Inc.) in children evaluated for possible sexual assault. All children in RI are referred to this one Child Safe Clinic. **Objective:** To report on the performance of the APTIMA COMBO 2 assay in combination with the individual FDA-cleared APTIMA GC and CT assays in identifying these STIs in children of possible sexual abuse. **Methods:** 438 children presenting at Hasbro hospital's ChildSafe clinic from October 2004 to February 2009 were tested. Urine samples were collected from each patient; genital, pharyngeal, and rectal swabs were collected when deemed clinically appropriate. Specimens were submitted for the APTIMA COMBO 2® GC/CT amplification assay. Positive samples were run in duplicate and subsequently tested with probe sets for alternate sequences (APTIMA GC and CT assays). **Results**: Children were 62.3% white and 54.5% pubertal with a mean age of 11.3 yr. Normal/non-specific findings were noted in the majority of patients (79.4%). Of 361 patients with confirmed or probable history of sexual abuse, 19 patients had STIs identified (5.6 %). 269 (74.5%) of population did not have previous consensual sexual experience and were 40.7% of the STI positive population. All positive NAAT urine results confirmed with the alternate probe NAAT as well as other specimen sites submitted for a sensitivity and specificity of 100%. **Conclusion:** APTIMA exhibited both excellent sensitivity and specificity in children being evaluated for possible sexual abuse. Urine in females showed 100% correlation with other invasive specimens. No false positive results were noted from any site. 100% correlation with original positive results was seen with the secondary APTIMA probe set. Neither physical findings nor disclosed contact with perpetrator were reliable in determining likelihood of patients that would be positive for STIs or which patients should be tested. Implications: APTIMA is an acceptable method for diagnosing GC/CT in child sexual abuse. Non-invasive urine specimens are preferred when genital infection is possible. Rectal/pharyngeal specimens should be considered when history suggests mucosal contact at these sites and may be important to determine the true STI incidence in boys. All patients presenting as probable victims of sexual assault should provide urine for screening regardless of physical exam findings or disclosed contact with perpetrator.

INTRODUCTION

Child victims of sexual assault are at risk for sexually transmitted infections (STIs) and the rate at which this occurs within a specific population is directly related to the prevalence in the perpetrating population. Infectious status may serve as evidence of assault in children with no disclosure of prior sexual activity; however traditional methods of evaluation for the presence of STIs are invasive and can be traumatic for the patient. Thus, there is much discussion in the literature about risk assessment and exclusion criteria for STI testing. More recent literature for detection of STIs in children of possible sexual assault has shown that nucleic acid amplification tests (NAATs) are superior for detection of Chlamydia trachomatis (CT) and/or Neisseria gonorrhea (GC), compared to current traditional culture methods from routine genital sites, even when non-invasive urine specimens were performed. In addition, NAATs could be used without compromising specificity. The use of a non-invasive and easily collected specimen in a traumatized population is not only desirable but tempers the reliance on exclusion criteria for testing prior to collection. Hasbro Children's Hospital's ChildSafe clinic in Providence, RI evaluates individuals under the age of 18 for possible sexual abuse and neglect. Being the only clinic of its kind in the state, all potential cases are referred to this one facility allowing for consistency in evaluation, testing, and treatment. A cooperative agreement between the Medical Directors of the ChildSafe clinic and the Microbiology laboratory as well as the district attorney of RI allowed initiation of routine testing by NAATs for detection of CT and GC with subsequent alternate target probe confirmation instead of culture beginning in 2004. The purpose of this study is to report on the performance parameters of the FDA-cleared molecular amplification assays (Gen-Probe APTIMA COMBO 2 and the APTIMA CT and GC assays in identifying the STIs, CT and GC in children of possible sexual assault and abuse over a 4 year period as well as to determine the prevalence of GC and CT in association with disclosed contact with perpetrator and physical exam findings in a low prevalence STI population.

MATERIALS AND METHODS

Study Setting and Population: The study took place at Hasbro Children's Hospital ChildSafe clinic in Providence, RI. The overall prevalence of CT and GC in the state of RI is considered low at 4.5 and 0.4, respectively. The study population consisted of a consecutive group of 438 individuals (358 girls and 85 boys) who presented at the ChildSafe clinic from October of 2004 to February of 2009. Potentially sexually victimized children and adolescents were referred to the clinic by their physician, social worker, or law enforcement professional. The study to review data related to this population and STI results was approved by the Institutional Review Board # 0063-08.

Data Collection: All presenting individuals and/or their guardians were subjected to an extensive interview by experienced faculty according to guidelines established by the American Professional Society on the Abuse of Children. Children also received a thorough physical examination conducted by physicians trained specifically in sexual abuse abiding established methods to determine if physical evidence of sexual contact existed, as well as identifying previous sexual contact.

Determination of Continued Assessment Under ChildSafe: After initial evaluation, which included collection of specimens, patients were subsequently categorized as possible/probable victims of sexual assault if there was clear disclosure of sexual assault[1] by patient and/or witness and/or examination revealed evidence suggestive of anogenital trauma or other physical findings implicated possible sexual contact (e.g. complete or partial transection of hymenal tissue, anogenital warts and/or ulcers).

Specimen collection: The routine specimens collected were urine, and/or a vaginal swab. Pharyngeal, and/or rectal specimens were collected when deemed clinically appropriate. All specimens were collected with Gen-Probe collection kits and identified as a Childsafe specimen and transported to the Lifespan Microbiology laboratory, Providence, RI, at room temperature.

Primary Screening Assay: Specimens were incorporated into the daily run of CT/GC screening by the APTIMA COMBO 2. The assay was performed and interpreted according to the manufacturer's package insert and as previously reported. The assay utilizes a unique target capture hybridization along with RNA transcription medicated amplification (TMA) with generation of a chemiluminescent signal produced as relative light units (RLUs). For child safe specimens, during the course of the study period, all specimens were performed in duplicate with the same RLU criteria above used for interpretation. Alternate specimen sites, pharyngeal and rectal, were validated by the laboratory, prior to testing any child safe specimens.

Alternate Confirmatory Probe Testing: Alternate probe testing was conducted with the APTIMA CT and GC assays on all Childsafe specimens determined to be positive by the primary screening assay. In addition, 100 randomly selected specimens, 50 for GC and CT respectively, determined to be negative for both infections by the primary screening assay were assessed with the alternate probe to determine the specificity of the alternate confirmatory probe. The Alternate probe targets and amplifies a different segment of the CT and GC organisms' RNA, respectively. Conducted in same manner as the primary screening assay, CT and GC alternate probe RLUs of 0 – 49, 50 to 100, and >100 were considered negative, equivocal, and positive respectively. A positive specimen was that which was positive in the primary screening assay in duplicate as well as positive with the alternate individual CT or GC probe in a subsequent assay.

Quality Control: Routine environmental sampling from 13 sites within and around the assay system continuously confirmed that amplicon contamination was not occurring. In addition, proficiency and control samples, using discard pharyngeal and rectal specimens, with and without added CT and GC, are routinely tested to monitor ongoing specificity and sensitivity limits of detection.

[1]Defined as any form of sexual contact without voluntary consent or when, because of mental deficiency s/he is incapable of giving consent or when s/he is below the arbitrary age of consent.

Statistical Analysis: Fisher's exact test was performed to calculate the statistical significance of associated risk factors using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Null hypothesis was rejected if p value was <0.05.

RESULTS

A final study population of 361 children and adolescents with a mean age of 11.2 years and standard deviation of 4.0 years was suitable for analysis. **Table 1** displays the study population demographics. Seventy-seven (17.5%) of the original 438 children were eliminated from the initial study population because they did not meet the criteria for probable sexual assault. Sixty-seven percent of those excluded were under the age of 11 with claims founded in custodial disputes; twelve percent were over the age of 14 and participating in consensual sexual relations sanctioned by RI state law. All eliminated children were negative for CT and/or GC.

Table 1: Demographic Characteristics of Study Population (n = 361)

Characteristics	Frequency	%
Age*		
0 to 2	3	0.8
3 to 6	62	17.2
7 to 10	67	18.6
11 to 13	95	26.3
14 to 16	119	33
17 to 18	15	4.2
Race/Ethnicity		
Black	44	12.2
White	223	61.8
Hispanic	23	6.4
Other	27	7.5
Not Provided	44	12.2
Gender		
Female	299	82.8
Male	62	17.2

*42.5% of Study Population was Prepubertal

Of the remaining 361 patients, 27 (7.5%) were positive for an STI. 94% of specimens collected were urine. The remaining 6% consisted of vaginal, rectal, or pharyngeal swab specimens collected as deemed clinically appropriate. All specimens positive for CT and/or GC by the primary screening assay were confirmed positive by alternate probe testing. No specimens tested in the equivocal range. A randomly selected group of one hundred specimens negative by the primary screening assay were also tested with the alternate probe assay and all were determined negative.

While the majority of patients assessed were females, 66 specimens were collected from 62 male victims of child sexual assault (17.2%); all were negative for STIs. Urine was the only specimen collected from 93.5% of boys. Rectal and/or pharyngeal specimens were collected in addition to urine from 6.5% of boys.

27 female patients were determined to be positive for an STI from 32 specimens. **Table 2** shows the prevalence of an STI relative to their age and disclosure of previous sexual history. 56.3% of positive girls had urine collected exclusively. 15.6% and 6.2% of positive girls also had positive endocervical and rectal specimens, respectively. All girls with positive endocervical swabs had positive urine.

Table 2: Prevalence Summary of STIs in 321 Sexually Assaulted Girls n(%) Based on Age and Previous Sexual History

	<14	≥14	Total
No Previous Consensual (n=210)	9(4.3)	2(1.0)	11(5.2)
Previous Consensual (n=111)	4(3.6)	12(10.8)	16(14.4)
Total (n=321)	13(4.0)	14(4.4)	27(8.4)

Table 3 shows the prevalence of STI results in the population based on the physical exam findings. 321 patients (88%) of the study population received a thorough physical examination conducted by physicians using established methods. 12% of the study population refused the physical examination. The exam was executed to determine if physical evidence of sexual contact existed. All patients, at minimum, submitted urine for STI testing regardless of completion of physical examination.

Table 3: STI (%) Prevalence in Population Based on Physical Exam Findings (n=361)

	Normal or Nonspecific (n=227)	Suspicious/ Suggestive (n=17)	Clear Evidence (n=77)	No Physical Exam Conducted (n=40)
Chlamydia trachomatis	2.8	-	2.2	2.2
Neisseria gonorrhea	0.3	-	-	-
Co-infection	-	-	-	0.3
Total	3.1	-	2.2	2.5

Table 4 shows the STI prevalence of all children determined by physicians to be probable victims of sexual assault regardless of degree of disclosed abuse and physical exam findings.

Table 4: STI (%) Prevalence in Population Based on Type of Sexual Assault Disclosed

	None Disclosed	Mucosal to Mucosal	Hand to Genital/ Fondling
No Previous Consensual (n=269)	0.4	3.0	0.7
Previous Consensual (n=92)	4.3	13.0	0.0

Table 5 shows the data pertaining to the perpetrator determined during patient record review. 72.7% of victims knew their perpetrator prior to the incident. Patients participating in consensual sexual activity not within the confines of Rhode Island state law (n=29 with infectious status in 20.7%) as well as minor patients retrieved after abandoning state care homes (n=13 with infectious status in 38.5%) were included in the study population.

Table 5: STI (%) Prevalence in Population Based on Disclosed Perpetrator

Perpetrator	STI (n)	% STI Within Total Population
Known by Victim Prior to Assault		
Family Member (n=94)	5	1.4
Extended Family Member (n=45)	1	0.3
Family Friend/ Babysitter (n=69)	4	1.1
Peer Classmate/Friend (n=33)	2	0.6
Total Known	12	3.4
Stranger/Unknown (n=78)	5	1.4

CONCLUSIONS:

- This is the first study providing data for a 4.5 year consecutive period assessing all child abuse victims from a single child abuse clinic with NAAT for determination of the STIs, CT and GC.
- Testing for CT and GC by non-invasive urine using the Gen-Probe APTIMA COMBO 2 and APTIMA CT and GC assays was highly reproducible, reliable and specific with no false positive specimens identified.
- STI prevalence was higher in children evaluated for sexual assault compared to the regional STI prevalence, including those children with no previous consensual sexual history.
- Statistically significant risk of infection was seen in non-white females and in those who had a previous sexual history, especially those who had abandoned state care.
- Females were the only positives with STIs in the patient population evaluated. Males were nearly 20% of patients evaluated, yet, based on patient disclosure an additional 14.5% and 58.1% of boys should have had pharyngeal and rectal specimens collected respectively. Data in MSM show that these sites are more likely to be positive than urine for STIs. Because these specimens were not obtained, no conclusion can be made regarding the prevalence of GC and CT in male child victims of sexual assault in our population.
- STIs were seen with normal or nonspecific findings and in patients unable to be physically examined as well as those with non mucosal-mucosal disclosures.

IMPLICATIONS:

- NAAT is both an acceptable and preferred method for testing for STIs in child victims of sexual assault.
- At minimum, Urine should be collected from all who present regardless of physical exam findings and disclosed contact with perpetrator.
- Individuals who disclose alternate site contact with perpetrator should also have specimens collected from those locations.
- Rigorous adherence to exclusion criteria for the purposes of identifying which patients require STI testing should be evaluated given the high reliability and ease and performance benefits of NAAT testing.