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Short Communication

Sexually transmitted infections in sexually abused children: an audit project to implement PCR tests in a child advocacy center in Türkiye

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ABSTRACT

Background. Sexual abuse in children can sometimes result in sexually transmitted infections (STIs), which can serve as crucial forensic evidence. Although PCR methods are now accepted as the gold standard for STI screening, they have not yet widely replaced traditional culture methods in Türkiye. This study aims to assess the necessity of implementing PCR-based STI testing at Child Advocacy Centers in Türkiye, where such testing is not routinely available.

Methods. Conducted between February and November 2023, this study included children who presented to the Child Advocacy Center of Marmara University Pendik Training and Research Hospital. High-risk victims were identified based on criteria including a history of penetrative sexual abuse and factors such as multiple perpetrators or significant age disparity. Serological tests and genital swabs were collected and analyzed using both bacterial culture methods and a comprehensive STI PCR panel.

Results. The study included 20 victims, with a median age of 16 years. STI PCR testing detected pathogens in 19 out of 21 samples, including *Chlamydia trachomatis* (20%) and *Neisseria gonorrhoeae* (5%). In contrast, culture methods identified no sexually transmitted pathogens.

Conclusion. PCR testing demonstrated significantly higher sensitivity for detecting STIs compared to traditional bacterial culture methods, as expected. Implementing PCR-based STI testing in Child Advocacy Centers is an urgent and essential need for providing an accurate diagnosis and robust forensic evidence, enhancing the care and legal protection of sexually abused children.

Key words: nucleic acid amplification test (NAAT), sexually transmitted disease, child monitoring center.

Sexual abuse encompasses a wide range of behaviors, ranging from inappropriate gestures to acts of penetration. Contrary to prevailing misconceptions, such abuse typically does not result in visible physical trauma on the victim's body. However, it has been observed that

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children who have undergone sexual abuse are increasingly susceptible to contracting sexually transmitted infections (STIs). These infections, which include *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum*, human immunodeficiency virus (HIV), and herpes simplex virus (HSV) type 2, present not only immediate health risks but also significant long-term morbidity and mortality if left untreated.[1] Moreover, their contagious nature renders them a pertinent public health concern. Importantly, the presence of such infections can serve as crucial evidence within legal proceedings of alleged instances of sexual abuse, sometimes constituting the sole substantiating evidence of the purported incident.

As healthcare professionals, our foremost priority is to identify and treat these infections, followed by investigating their origin, particularly inquiring about the possibility of sexual abuse. Furthermore, in instances where allegations of sexual abuse arise, it is incumbent upon us to elucidate the significance of these findings to the judicial system and, as appropriate, meticulously preserve pertinent samples as evidence for subsequent submission to law enforcement agencies. The identification of infections caused by *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, HIV (in cases where transmission through blood or contaminated needles has been ruled out), and *Treponema pallidum* among individuals under the age of 16, particularly when perinatal transmission has been discounted, serves as compelling evidence of sexual abuse (the provisions outlined in the current Turkish Penal Code, which stipulates the age threshold at which a child can legally consent to sexual intercourse as 16 years.).[2-4]

The nucleic acid amplification test (NAAT), commonly known as the PCR test, has been accepted as the gold standard instead of the traditional culture method for diagnosing sexually transmitted infections, exhibits considerably higher sensitivity.[5] The Centers for Disease Control and Prevention (CDC) has embraced the NAAT as a diagnostic tool, supplemented by a confirmatory NAAT targeting distinct genetic regions within the bacterial strains, thereby supplanting the conventional reliance on culture-based methods.[6]

Child Advocacy Centers (CACs) in Türkiye currently lack available STI PCR testing for routine use with sexually abused children. With this audit Project, we aimed to assess the necessity of STI PCR tests in CACs in Türkiye.

Materials and Methods

The study was performed according to the World Medical Association Declaration of Helsinki principles. It was approved by the local ethics committee of the Medical Faculty of Marmara University (Protocol No: 09.2023.196). The study included children who applied to the CAC of Marmara University Pendik Training and Research Hospital, Türkiye between February and November 2023. They were recruited with the informed consent of both the child and their legal guardian (usually one of the parents) before the anamnesis. The study involved high-risk victims to assess the need for incorporating STI PCR testing into routine procedures at CACs. Criteria to identify high-risk victims were established based on the literatüre.[2,7,8] Victims in the high-risk group are identified by a history of penetrative sexual abuse along with one or more of the following factors: multiple perpetrators, vaginal discharge after the incident, substance use, significant age disparity (more than 5 years), or lack of familiarity between the child and the perpetrator. The age of the participants, their habits (such as smoking and alcohol use), medication use, number of sexual partners or assailants, and physical examination findings were all assessed.

Serology

Serology tests including Anti-HBC IgG, Anti-HBC IgM, Syphilis antibody, Anti-HBS, HBsAg, Anti-HIV1/2+p24 Ag, Anti-HCV, and VDRL-

RPR have been quantified from all victims who consented to give blood samples.

Molecular methods

Genital samples from all participants were collected from the vagina using Dacron swabs (Bioeksen, İstanbul-Türkiye). Following collection, the samples were immediately transported and stored at -80°C to preserve them until molecular testing. On the day of the study, samples were retrieved from the -80°C freezer and thawed at room temperature for approximately 30 minutes. Each sample was then vortexed for 15 seconds to ensure homogeneity.

DNA extraction was automated using the rapid nucleic acid isolation kit (Bioeksen, Istanbul-Türkiye) on a Zybio EXM 3000 instrument. Subsequently, the Bio-Speedy Sexually Transmitted Infections RT-qPCR Panel Kit (Bioeksen, Istanbul-Türkiye) was employed to qualitatively assess the presence of specific pathogens in the extracted DNA. According to the manufacturer's instructions, this kit utilises reverse transcription and real-time multiplex PCR (RT-qPCR).

HSV-1, HSV-2, *Mycoplasma genitalium*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Ureaplasma parvum/urealyticum, Mycoplasma hominis, Haemophilus ducreyi, Streptococcus agalactiae, Gardnerella vaginalis* were screened from vaginal swabs by PCR test.

Bacterial culture methods

Swabs submerged in Amies medium (True Line, China) underwent bacterial culture and Gram stain preparation. All samples were inoculated onto three different media: Columbia agar with 5% sheep blood (bioMérieux, France) for general bacterial growth, MacConkey agar (bioMérieux, France) to differentiate lactose-fermenting from non-fermenting bacteria, and selective chocolate agar (PolyViteX VCAT3, bioMérieux, France) for fastidious organisms. Any identified growth was further analyzed using MALDI-TOF MS (VITEK MS, bioMérieux, France) for definitive bacterial identification.

Genital cultures, and specimens for STI PCR panels were collected from all children meeting the inclusion criteria on the day of admission to the CAC. Regardless of the test results, all victims were administered prophylactic treatment consisting of a single intramuscular dose of ceftriaxone 500 mg, oral doxycycline 100 mg twice daily for 7 days, and oral metronidazole twice daily for 7 days as per guidelines.[3]

Results

During that period, 638 applications were submitted, and 219 of them were physically examined. A total of twenty victims, encompassing twenty-one separate assault events, were included in the study. The median age of the victims was 16 years (25th percentile: 14 years, 75th percentile: 17 years). Upon examination of the victims' medical histories, a history of smoking was identified in 65% (n=13) of cases, alcohol use in 55% (n=11), use of antidepressant/antipsychotic medications in 55% (n=11), and past or present use of substances in 35% (n=7). Multiple sexual intercourses were experienced with more than one partner before the examination in 90.5% (n=19) of the cases. An age disparity exceeding five years between victim and perpetrator was observed in 47.6% (n=10) of cases. Notably, in 81% (n=17) of the incidents, the perpetrator was unknown to the child. A history of incest was documented in two assaults. Four victims (20%) complained of vaginal discharge. Thirteen victims (65%) had a genital traumatic lesion.

Nineteen out of 20 victims consented to blood sampling. All tested participants exhibited negative test results except anti-HBs marker. Eleven participants had negative results for anti-HBs, prompting the scheduling of hepatitis B vaccination.

The median interval between the assault event and the admission was detected as 12 days (25p: 0 day, 75p: 49 days).

Table I. The summary of the results.

Case number	Vaginal discharge	Medical findings	Serologic tests	Vaginal culture	Vaginal PCR
1	+	Healed hymenal transection	Anti-HBS +	*Candida albicans* +	*Gardnerella vaginalis, Haemophilus ducreyi, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
2	+	Healed hymenal transection	Anti-HBS +	*Candida albicans* +	*Gardnerella vaginalis, Haemophilus ducreyi, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
3	-	Healed hymenal transection	-	Normal flora	*Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
4	-	-	Anti-HBS +	Normal flora	*Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
5	-	Healed hymenal transection	Anti-HBS +	Leukocyte +, Normal flora	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
6	+	-	-	Normal flora	***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
7	-	Healed hymenal transection	-	Leukocyte +, *Candida albicans* +	***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
8	-	Healed hymenal transection	-	Leukocyte +, Normal flora	*Gardnerella vaginalis, Haemophilus ducreyi, Ureaplasma parvum/urealyticum*
9	-	Healed hymenal transection	-	Normal flora	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
10	-	Healed hymenal transection	-	Leukocyte +, Normal flora	*Gardnerella vaginalis*
11	-	-	Anti-HBS +	Normal flora	***Neisseria gonorrhoeae***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
12	-	-	Anti-HBS +	Leukocyte +, Normal flora	*Ureaplasma parvum/urealyticum*
13	-	Healed hymenal transection	-	Normal flora	***Chlamydia trachomatis***, *Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
14	-	Healed hymenal transection	Anti-HBS +	Normal flora	-
15	+	-	-	Normal flora	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
16	-	-	-	Normal flora	***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum*
17	-	Healed hymenal transection	Anti-HBS +	*Candida glabrata* +	*Ureaplasma parvum/urealyticum*
18	-	Healed hymenal transection	X	Normal flora	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
19	-	Healed hymenal transection	-	*Staphylococcus aureus* +	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
20	-	-	-	Normal flora	*Gardnerella vaginalis, Ureaplasma parvum/urealyticum*
21	-	-	Anti-HBS +	Normal flora	-

Note: "+", detected; "-", not detected, "x", not taken. Case 4 and Case 11 are the same person but involved distinct incidents.

Genital swab culture yielded *Candida albicans* in 3 samples, *Candida glabrata in 1 sample,* and *Staphylococcus aureus* in 1 sample. However, no sexually transmitted infections were detected in any victim by culture.

PCR assays identified *Chlamydia trachomatis* in four victims with cycle threshold (CT) values of 19.2, 20.6, 23.5 and 24.0, respectively. *Haemophilus ducreyi* was detected in three victims with CT values of 17.6, 23.1, and 28.0, and *Neisseria gonorrhoeae* was identified in one victim with a CT value of 14.6. The overall results are summarized in Table I. Positive and negative control samples were run, and all results are shared in the supplementary data.

Discussion

This study reaffirmed that the PCR method exhibits greater sensitivity and specificity compared to conventional culture methods. The incorporation of STI PCR testing is crucial in the management of sexually abused children and should be implemented in all CACs.

While no sexually transmitted pathogens were identified using cultures, PCR identified at least one pathogen in 19 out of 21 samples. This finding aligns with previous research.[5] These PCR-identified pathogens do not necessarily indicate an active infection. However, the CT threshold may inversely reflect the pathogen loads. Four cases (20%) had *C. trachomatis*, and one case (5%) had *N. gonorrhoeae*. Similar to other studies, *N. gonorrhoeae* prevalence was lower than *C. trachomatis*.[9] The detection of the STI pathogen rate in our study was higher than in other studies[10-12], which is thought to be due to participants having high-risk factors. We did not find a statistically significant difference in detecting STI pathogens between the group with traumatic genital findings and the group without. It is important to note that the sample size of this study was small for generalization, and all participants were already in the high-risk group for STIs.

Even though the participants were postpubertal children, the prepubertal children are admitted to the CAC. The prevalence of STIs is lower in prepubertal children compared to postpubertal. Standard culture methods lack sensitivity, and there is also a lack of data on the sensitivity and specificity of NAATs in the prepubertal age group.[13,14] These limitations pose extra challenges for accurate diagnosis and management of STIs in prepubertal children. We also should keep in mind that NAAT can detect bacteria without clear clinical significance, so cautious medical interpretation is required.[11]

A case involving two separate examinations is presented, prompted by a second criminal act committed three and a half months following the initial incident. The first PCR test for *N. gonorrhoeae* yielded a negative result, while the subsequent PCR test conducted during the second examination returned a positive result. Notably, the initial incident transpired three months before the first examination, while the second incident occurred only five days before the second examination. This finding has the potential to serve as evidence of sexual assault of the second crime, contingent upon a thorough investigation into the chronological details surrounding both incidents.

In three cases, despite the absence of any anogenital traumatic injury, PCR detected *C. trachomatis* in two cases and *N. gonorrhoeae* in one case. These infections constituted crucial physical evidence of alleged sexual abuse. Using culture methods alone would not have identified these infectious agents, underscoring the importance of STI screening at CACs. Given the potential legal ramifications of positive results, microbiology laboratories must retain both samples and isolates. Furthermore, authorities should establish and approve PCR screening and confirmatory tests, such as those endorsed by the FDA, if not already in place.[14]

Culture is not a diagnostic assay like PCR; hence, there exists a possibility of cultivating an agent in culture that may not be detected by the PCR test.[15,16] For this reason, and sensitivity

and specificity issues, the CACs must start to use PCR tests for STI, but also might continue to take culture samples along with PCR tests.

The updated guideline now recommends that STI testing should not be restricted solely to sites where penetration is described. This is due to the possibility of incomplete disclosures by the child and the potential for contiguous spread from the genitals to the anus, particularly in females. Confirmatory testing of positive results is recommended, particularly in situations where the findings may have legal significance, such as in children who are under 16 or sexually non-active (according to the Turkish Penal Code, it is currently in force). Pharyngeal *N. gonorrhea* and *C. trachomatis* have been added to the "Infections caused by sexual contact, if confirmed by appropriate testing, and perinatal transmission has been ruled out" section of the recent guideline.[2] Testing urine samples from boys is also recommended in the literature.[17] However, we exclusively collected vaginal samples from the children, as the PCR test kits utilized in this study are validated solely for vaginal swabs.

The major limitation of our study is the small sample size, which impeded the attainment of statistically significant results. Additionally, the absence of a control group consisting of non-sexually abused children further constrained the study's robustness. Another significant limitation was the lack of a confirmatory NAAT targeting different regions of the genetic material of the infectious agents, which could have validated the initial test results.

Our study reconfirmed that culture techniques are insufficient for the detection of STIs in sexually abused children. This audit highlights the imperative need for the implementation of PCR testing in CACs across Türkiye. Such measures not only enhance diagnostic accuracy but also provide robust forensic evidence for legal proceedings.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.5188

Ethical approval

The study was performed according to the principles of the World Medical Association Declaration of Helsinki. It was approved by the local ethics committee of the Medical Faculty of Marmara University (Protocol No: 09.2023.196).

Author contribution

Study conception and design: ST, ZE, MY, ES, TK, FHU, ZAİ, NÜT, EKK, MAİ; data collection: ST, TK, MY, ES; analysis and interpretation of results: ST, ZE, TK, MY, ES; draft manuscript preparation: ST, ZE, TK. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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