Int J Legal Med (2005) 119: 158–163
DOI 10.1007/s00414-004-0503-y

TECHNICAL NOTE

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Y-STR analysis for detection and objective confirmation of child sexual abuse

Received: 21 April 2004 / Accepted: 12 October 2004 / Published online: 24 November 2004

Abstract We evaluated 26 child sexual assault cases for the incorporation of Y-STR screening in the routine detection and objective confirmation of sexual contact between the child victim and the perpetrator. Various samples, e.g. vaginal or anal swabs from patients aged 2–17 years old (25 females, 1 male), were collected 6–72 h after the incident. Due to the limited amounts of DNA in these samples, total DNA was extracted using a one-step procedure and screened with autosomal STRs to detect signs of a victim-assailant DNA mixture and with Y-STRs for assailant DNA. Autosomal STRs failed to give signs of victim-assailant DNA mixtures while Y-STRs were detected in 24 of the 26 cases corresponding to a success rate of 92.3%. With the possible presence of both male sperm and/or male epithelial cells in forensic evidence, Y-STR DNA markers were detected regardless of external ejaculation, microscopic detection of sperm and with post-coital intervals of up to 72 h. While only partial profiles were generated owing to low quantities of male DNA present, Y-STR screening results can serve as objective evidence of sexual contact in child sexual abuse cases involving victims who do not have any previous sexual history. This type of evidence can corroborate child victim testimony and spare the child victim from further trauma caused by prolonged forensic investigations and court proceedings. Alternatively, Y-STR screening can provide objective proof of non-involvement of an accused with the victim.

Keywords Y-STR analysis · Philippines · Child abuse · Child protection unit · Objective confirmation

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Introduction

Sexual assault is considered a crime of violence and in the Philippines it is classified as a "heinous crime" punishable by a sentence ranging from life imprisonment (*reclusion perpetua*) to death. A mandatory death sentence is meted when victims are below 7 years old or when victims are below 18 years of age and the offender is related within the third degree of consanguinity [1]. Victims are usually women and children, while assailants are nearly always men [4] and in child sexual abuse cases in the Philippines, 99.9% of offenders are male (http://www.childprotection.org.ph).

General guidelines for forensic evidence collection in sexual assault cases exist, but are based on cases involving adult victims. Compared to adult victims, there is a need for specific guidelines when dealing with prepubertal victims due to variations in physical make-up and capacity to deal with trauma. Children who are in a state of trauma may not provide a complete description of the assault and may refuse to cooperate during the collection of biological samples [3].

In the Philippines, a considerable number of child sexual abuse incidents occur. From 1997 to 2000, a total of 2,243 child abuse cases were reported to the Child Protection Unit, Philippine General Hospital, Manila, while many more remain unreported (http://www.childprotection.org.ph). Approximately 72% (1,609) of these reported cases involved sexual abuse. Child protection units located in different cities provide a multidisciplinary approach for a comprehensive medical examination, treatment and psycho-social services to abused children and their families. Physical examination of child patients includes collection of biological samples such as body swabs which may be used to detect non-patient material (e.g. sperm cells, semen) to confirm that sexual contact did occur. However, medical examiners have observed delays in the reporting of incidents (>72 h) and a relative low success rate of sperm detection using microscopy. Other than the loss of viability of sperm, a variety of events (e.g. bathing, disposal of clothing etc.) may occur during these delays that could lead

to the loss of valuable biological evidence. Another area of concern is that the majority of convictions in child sexual abuse cases are based on testimony [1]. Reliance on testimony may cause additional trauma to child victims who are made to recount their ordeal in prolonged litigations [1] or may lead to erroneous convictions [5, 16]. Hence there is a need for a sensitive and objective tool to assist investigations of child sexual abuse.

With the rapid advancement in forensic DNA technology, DNA analysis of biological samples offers an additional means of investigating child sexual assault cases. Y-chromosome short tandem repeats (Y-STRs) that exclusively target male DNA have been used in sexual assault investigations [9, 11, 12, 14, 15, 17, 19]. Y-STR typing simplifies interpretation of DNA typing results since the female victim's DNA is not observed. In the case of male prepubertal victims, the presence of male DNA mixtures provides information of possible sexual contact with a male perpetrator. Y-STR analysis also facilitates a better estimate of the number of male assailants than autosomal STRs, provided these individuals are unrelated [9, 11, 12, 14, 15]. We report here the evaluation of a Y-STR screening procedure in 26 child sexual abuse cases for subsequent incorporation into routine forensic examinations.

Materials and methods

Samples

Within the initial year of collaboration (January 2002–January 2003) between the Child Protection Unit, Philippine General Hospital and our laboratory, samples from 26 child sexual abuse cases handled by the Child Protection Unit were analyzed. These 26 cases were the only cases reported within 72 h of the incident. Case background information such as victims' age, sex (25 female, 1 male) and sperm detection data (Table 1) were provided by the Child Protection Unit. All procedures performed by the Child Protection Unit are non-traumatic to child patients and adhere to all legal requirements. All patients and/or their legal guardians involved gave their informed consent prior to inclusion in the study. The cases involved patients 2–17 years old who did not have any previous sexual history. Vaginal/anal swabs from the patients were collected by medical examiners within 72 h of the incident. Calcium alginate ("calgi") swabs were used for patients below 15 years old, while standard cotton-tipped pledgettes were used for older patients (Fig. 1). Oral swabs were also collected from victims as a source of reference DNA. All samples were divided into two equal parts, one for DNA analysis and the other for future reference.

Table 1 Summary of background information for CPU sexual assault cases

Case	Victim's age (years)	Collection time (hours)	Ejaculation	Sperm detection
1	16	11	Internal vaginal	-
2	6	24	Undetermined	nd
3	12.5	18	Undetermined	-
4*	16	24	Internal vaginal	+
5♂	6	24	Internal anal	+
6	15	9	Undetermined	-
7	16	30	Undetermined	+
8	8.5	23	No ejaculation	-
9	15	51	Internal vaginal	-
10	ni	48	Internal vaginal	nd
11*	14	24	Undetermined	nd
12	13	22	No ejaculation	-
13*	14	21	Undetermined	+
14	15	24	No ejaculation	+
15	15	11	No ejaculation	-
16	17	12	Undetermined	nd
17	12	8	Internal vaginal	nd
18	2	6	Internal anal	nd
19	12	16	No ejaculation	nd
20*	14	19	undetermined	-
21	ni	ni	ni	nd
22	14	14	Undetermined	-
23	10	72	ni	nd
24	17	45	Undetermined	nd
25	14	14	No ejaculation	-
26	14	17	Undetermined	nd

(−) Negative for sperm, (+) positive for sperm.
*nd*Test not done, *ni* no information given.
(♂) The only case involving a male victim.
*Cases reportedly involving multiple assailants.

DNA extraction

A one-step DNA extraction procedure (without separation of male and female fractions) modified from a two-step differential lysis FBI protocol [6] was used to extract DNA from vaginal and anal swabs. Briefly, each cotton tip was moistened with 150 µl of sterile distilled deionized water in a 1.5 ml microfuge tube. A sterile scalpel blade was used to separate the cotton tip and the wood (wooden pledgette) or metal ("calgi swab") applicator stick. The cotton tip was treated with lysis solution containing 150 µl TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl), 50 µl Sarkosyl (20%), 7 µl DTT (1 M) and 10 µl proteinase K (20 mg/ml) at 56°C for 18–24 h. Lysates were subjected to standard phenol extraction [6] and DNA was further purified using Microcon100 DNA concentrators (Amicon, MA) following the manufacturer's instructions. DNA from reference oral swabs was extracted using a standard organic extraction method [6]. DNA was quantitated using the QuantiBlot human DNA quantitation kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

DNA amplification and fragment analysis

Individual locus (singleplex) amplification of 5–50 ng DNA at the autosomal STR loci HUMTH01 and HUMvWA

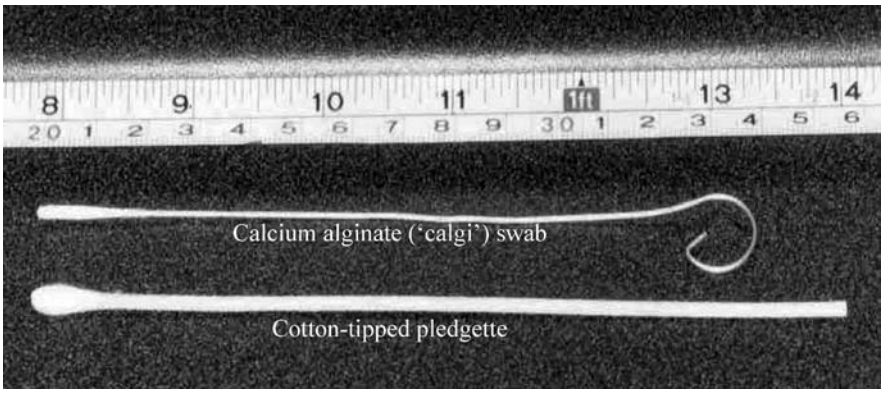

Fig. 1 Swabs used for collection of sexual assault evidence

was performed as described by Halos et al. [10]. Singleplex Y-STR amplification at the DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 loci was performed as described by Kayser et al. [12]. Fragment analysis was performed using the ALFexpress DNA sequencer with AlleleLinks software (Amersham-Pharmacia Biotech, Uppsala, Sweden) using in-house ladders [10, 13, 18]. All allele assignments conformed to published nomenclature and the guidelines for Y-STR analysis set by the International Society of Forensic Genetics (ISFG) [8, 13].

Results and discussion

The presence of spermatozoa detected via microscopy is most often sought as evidence from sexual assault victims and its absence or loss (after 72 h) can lead to the curtailment of forensic biological investigations [2, 4, 17]. The Child Protection Unit conducts microscopic sperm detection only for cases reported within 72 h of the incident. Beyond this period of sperm viability, body swabs from the child victim are not collected in order to prevent further trauma and discomfort. In these situations, evidence of sexual assault is obtained from results of medical examination. The observation of sperm in the genitalia of a child who has no previous sexual history is conclusive evidence of sexual contact [7]. As a screening tool to establish sexual contact, the detection of male DNA using Y-STRs can be comparable to detection of sperm [2]. Hence to compare the sensitivity of Y-STR screening with microscopic detection of sperm we only considered cases where biological sampling was conducted within 72 h of the reported incident.

In DNA analysis of sexual assault evidence, differential lysis is a method commonly used to separate male (assailant) sperm and female (victim) epithelial cell DNA. Initial lysis of female epithelial cells leaves sperm intact for effective separation of DNA fractions. Notably, male ejaculates contain components, (e.g. epithelial and inflammatory cells) other than sperm cells, hence differential lysis may not completely separate male and female fractions due to male epithelial cell lysis [4, 19]. Sperm may also lyse prematurely [9, 11, 19]. Furthermore, multiple steps in the differential lysis method may lead to loss of evidentiary DNA [9, 11]. This can be circumvented by a one-step DNA extraction procedure that eliminates the separation of male and female components resulting in a purified mixture of male-female DNA. Subsequent analysis can be done using Y-STRs that specifically target male DNA [9, 11, 12, 14, 15, 17, 19]. Anticipating the possibility of low recovery of biological evidence from the assailant, this study used a one-step DNA extraction method followed by Y-STR and autosomal STR screening.

Autosomal STR screening using markers HUMTH01 and HUMvWA did not provide any indication of DNA mixtures. In all 26 cases, only the autosomal STR profile of the child victim was observed. In contrast, Y-STRs detected male DNA in 24 out of the 26 cases, corresponding to a 92% detection rate compared to 19% for microscopic examination. Partial Y-STR haplotypes were generated due to low amounts of male DNA (Table 2) that provided sufficient proof of the presence of non-victim DNA. The presence of foreign male DNA in the genitalia of child victims who do not have any previous sexual history corroborates testimonies of sexual contact. This evidence is important for social workers to assist child victims and to pursue the progression of these cases in courts of law. In the absence of DNA legislation in the Philippines, a court order is required for the collection of a suspect's reference sample for a complete Y and autosomal DNA analysis.

Varying rates of positive detection were observed for different Y-STR loci in our singleplex reactions (Table 2) that are marker specific. In the absence of instrumentation for commercially available multiplex Y-STR systems, we suggest the use of more sensitive Y-STRs such as DYS390, DYS19, DYS389I, DYS389II and DYS393 for initial Y-STR screening of child sexual assault evidence.

Based on information provided by the child victims, 81% of these cases involved single assailants which were consistent with data generated using Y-STR haplotyping. In all four cases where multiple assailants were reported (cases 4, 11, 13 and 20), more than one signal was observed at one or more of the loci DYS19, DYS389I, DYS389II and DYS393 (Table 2). Figure 2 shows an example for case 4. Using the current Philippine Y-STR database with a power of discrimination of 0.9996 [13, 18], Y-STR haplotyping can readily distinguish between multiple assailants provided they are unrelated. However, cases involving related assailants present problems for Y-STRs. In case 11, the victim claimed that she had been assaulted by three in-

Table 2 Summary of Y-STR results for sexual assault samples

Case	Y-chromosome STR							
	Y19	Y389I	Y389II	Y390	Y391	Y392	Y393	Y385
1	-	-	-	-	-	-	-	-
2	-	11, 16	-	27	-	-	-	14
3	-	11	-	27	-	-	-	13
4*	15, 16	12, 13	27, 28	22	-	-	12, 13	14
5♂	-	-	-	23	-	-	-	-
6	15	-	-	27	-	12	13	13
7	15, 17	11	-	24, 26, 27	-	12, 14	11, 13	15
8	17	11	-	24	-	12	12, 13	13
9	-	-	-	25, 27	-	-	-	-
10	15	12	28	23	10	12	12	-
11*	15	11, 12	28	23	10	15	13	-
12	-	-	-	-	-	-	-	-
13*	15	12, 13	31	24	-	11	12	-
14	15	-	-	24	-	13	-	-
15	17	11	-	27	-	12	12, 13, 14	-
16	-	-	-	24	-	-	-	-
17	15, 16, 17	11	-	26	-	-	-	-
18	-	11, 13	29	27	-	-	-	-
19	17	-	-	25	-	-	13	-
20*	14, 15	12, 13	28	24, 25	-	13	12, 13	-
21	17	-	-	26, 27	-	-	-	-
22	-	-	-	27	-	-	-	-
23	-	11	-	27	-	-	-	-
24	-	-	-	24	-	-	12	-
25	16	13	29	23	-	-	13	-
26	15	12	27	23	-	-	13	-
SR%	61.5	57.7	30.8	92.3	7.7	34.6	50	23.1

(−) No result, *SR*%, success rate in percentage.
(♂) The only case involving a male victim.
*Cases reportedly involving multiple assailants

dividuals, however, Y haplotyping was consistent with two male sources. Further investigation revealed that two of the individuals whom the victim identified were paternal cousins and would therefore have (except in case of mutation) identical Y-STR haplotypes. In another case (case 7) more than one signal was observed at DYS19, DYS390, DYS392 and DYS393 consistent with more than one male source although the victim reported only a single assailant. Con-

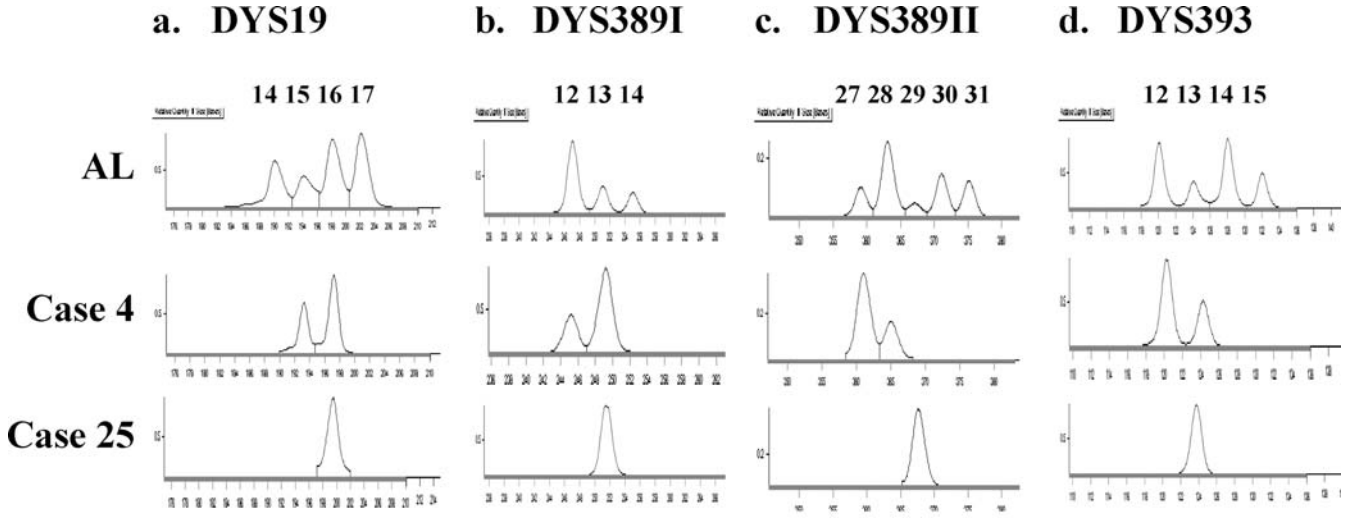

Fig. 2 Column headings (*a* DYS19, *b* DYS389I, *c* DYS389II and *d* DYS393) refer to Y-STR loci. The row labeled *AL* shows the allelic ladders for each locus and their corresponding allele designations. *Rows* labeled case 4 and case 25 show results at each locus for CPU case 4 and CPU case 25, respectively. For a vaginal swab from a case (case 4) reportedly involving multiple assailants, the number of Y-STR peak signals (2 peaks) generated corresponded to the reported number of male assailants (2 assailants). The vaginal swab from a CPU case (case 25) reportedly involving a single assailant generated a single Y-STR peak signal

tamination by the analyst was ruled out since his DNA profile was not the same as that observed in the evidentiary sample. This information was provided to the social worker dealing with the child victim for further verification.

Investigations into child sexual assault cases can be very difficult because children may not provide a complete description of the assault and may be uncooperative during sample collection. Initially, doctors only collected biological samples if child victims reported their assailants to have ejaculated. In the present study, we were able to generate male DNA profiles in cases where there was no report of internal vaginal and/or anal ejaculation (cases 8, 12, 14, 15, 19 and 25). Figure 2 shows an example for case 25. Effectiveness of Y-STR screening for confirmation in child sexual abuse cases is further demonstrated in cases where ejaculation could not be physically determined (cases 2, 3, 6, 7, 11, 13, 16, 20, 22, 24 and 26) as well as cases where no information could be derived (cases 21 and 23).

Other than the lapse of the 72 h period of viability, the absence or loss of detectable sperm cells in samples collected from a victim may be attributed to various factors ranging from the assailant's physiology to the victim's actions after assault [4, 9, 11, 17]. However, male DNA from lysed sperm cells or male epithelial cells can still be detected. This study shows that Y-STR screening was positive for the presence of male DNA whether sperm is detected (positive Y-STR results for 5 out of 5 cases), not detected (positive Y-STR results for 6 out of 8 cases) or where simply no determination was conducted (positive Y-STR results for 13 out of 13 cases). Y-STR screening therefore provides objective evidence of sexual contact in child abuse cases involving victims who do not have any previous sexual history. In addition, we recommend the use of thin calgi swabs (Fig. 1) for the collection of biological samples from the child's genitalia to decrease the discomfort experienced by child victims.

Male DNA was not detected by Y-STR screening in cases 1 and 12. While the victim reported internal ejaculation for case 1, sperm were not detected. Background information revealed the victim was menstruating and had showered after the assault, thereby losing biological evidence. In case 12, the reported non-ejaculation of the assailant was consistent with the absence of male DNA. However, this does not rule out abuse and other sources of evidence such as medical examination and the victim's account must be evaluated.

In case 5, which involved a male child victim, a single Y-STR allele at DYS390 consistent with the child's DNA was generated from an anal swab. Although the child reported internal anal ejaculation, amplification at other Y-STR loci was unsuccessful possibly due to the low amount of DNA in the evidentiary sample.

Due to the sensitive nature of child abuse cases, the Child Protection Unit employs a non-disclosure policy which controls the release of any information. For the 26 cases reported here it was permitted to report that 1 case had an amicable settlement, 6 cases were filed and have reached the court, 5 cases await verdict, 13 cases have yet to be filed and 1 case did not progress. Suspects in these cases were identified based on testimony and obtaining reference samples from these individuals for DNA typing still requires a court order.

The status of these 26 cases reflects the slow progression of child abuse cases which unfortunately is a trend for the majority of such cases in the Philippines. This long process entails the child victim's testimony several months to more than a year after the actual incident occurred. For the child victim it is difficult, even traumatic to recall the ordeal after such time. Furthermore, during the delay, inconsistencies or even retraction of testimonies may occur. The availability of DNA evidence in the form of Y-STR screening results provides objective evidence that can support the child victim's testimony throughout the duration of the case.

In this study, we have demonstrated the sensitivity of Y-STRs in the preliminary screening of forensic samples from child sexual abuse cases. Notably, whilst Y-STR haplotyping of a forensic sample without a comparison to a reference sample from a suspect can only provide information about the presence or absence of male DNA, the detection of male DNA in the child's genitalia is conclusive evidence of sexual contact in child abuse cases involving victims who do not have any previous sexual history. The initial Y-STR screening results from the forensic sample may then be used for subsequent matching with a reference sample from a suspect obtained through the appropriate legal procedure. The male specificity of the Y-STR system complements the use of a one-step DNA extraction procedure that has fewer steps than differential extraction protocols thus preserving important evidentiary DNA samples for subsequent amplification. DNA from the remaining 50% of forensic case samples that have been stored may then be extracted using differential lysis (two-step) procedures for complete autosomal DNA typing for which matching probability statistics are well-established. Although appropriate statistics for the use of Y chromosomal DNA evidence in court are still being evaluated, the availability of physical evidence of sexual contact in cases of child victims is a major step that can accelerate the investigation, the progression of the case in court, and subsequent rehabilitation of the child.

Lastly, but of equal importance, is that there should be equal awareness that an innocent individual may be accused of sexual assault [4]. In Philippine courts, the majority of convictions of child sexual abuse have relied on a child's testimony which at times is found to be false [5, 16]. In the Philippines where a mandatory death penalty is meted out to individuals convicted of child sexual abuse, the use of DNA technology as an objective tool to assist in identification of the real perpetrator and exclusion of the innocent accused, is a step in the right direction.

Conclusions

Y-STR analysis is a powerful tool for the detection of male DNA in evidentiary samples collected from child victims of sexual abuse. Specifically, in child abuse cases, the presence of male DNA in the genitalia of a child victim who has

no previous sexual history is a clear indication of sexual contact. While there will be temporary discomfort for victims during biological sampling, this is outweighed by the advantages of obtaining objective physical evidence that can corroborate testimony. The availability of DNA evidence can spare the child victim from further trauma caused by repeated testimonies and long court trials. For the accused on the other hand, Y-STR screening can be an objective tool to prove possible involvement or innocence. This study has provided a basis for a national strategy to incorporate Y-STRs in routine screening of physical evidence from child sexual abuse cases handled by various child protection units across the country.

Acknowledgements This study was supported by the European Commission, the Philippine Council for Advanced Science Research Technology and the Office of the Vice-Chancellor for Research and Development of the University of the Philippines, Diliman. The contents of this paper do not necessarily represent the views of the European Commission. The authors also acknowledge Dr Raquel Fortun for interesting insights during the study.

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