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Original communication

Urine specimen collection following consensual intercourse – A forensic evidence collection method for Y-DNA and spermatozoa

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ABSTRACT

The purpose of the prospective research was to evaluate the benefit of urine specimen as a collection technique for biological forensic evidence in adult volunteers following consensual intercourse. For detecting Y-chromosomal material Buccal Swab Spin Protocol® was used in DNA extraction and purification and samples were analysed with Quantifiler Y Human Male DNA Quantification Kit®. The time frame for positive Y-DNA was evaluated. Immediate microscopy for detection of spermatozoa was performed.

Y-DNA was detected in 173/205 (84.4%) urine samples. Of the 86 first post-coital void urine samples available, Y-DNA was detected in 83 (96.5%) specimens. Of the 119 urine samples from volunteers with post-coital activities Y-DNA was still measurable in 70 (58.8%) urine specimens. The male DNA amount was below 0.023 ng/μl in 28/153 (18.3%) urine samples.

Of the 22 urine samples obtained after 24 post-coital hours, 9 (40.9%) were still Y-DNA positive.

No associations were found between coital durance, coital frequency during the past two weeks prior to the study intercourse, post-coital activities, and the urine sample Y-DNA positivity.

Of the 111 urine samples where the immediate microscopy was performed, in 66 (59.5%) samples spermatozoa were verified and one sample even contained motile spermatozoa. Microscopy detected 66 (67.3%) and failed to detect spermatozoa in 32 (32.7%) of Y-DNA positive samples.

In addition to conventional invasive swab techniques, urine samples seem to be an effective biological trace collection method for Y-DNA and spermatozoa within 24 h following penile-vaginal penetration. Furthermore, it may be considered as a non-invasive collection method in suspected acute child sexual abuse cases to diminish time delay in forensic evidence collection and to improve patients' positive attitudes towards evidence collection.

1. Introduction

In suspected sexual assaults biological trace evidence is most often collected by swabs from the anogenital area. The possibility of using urine for evidence collection has rarely been considered.

The idea of urine sample collection for forensic purposes is based on human anatomy and physiology. In women, the urethral orifice in the vulvar area is next to the hymenal opening of the vagina. During voiding, the vaginal outflow of secretions increases due to physiologic relaxation of the pelvic floor and due to the increase in abdominal pressure. The urine rinses the periuretral vulvar area and the vaginal orifice and it can be collected for further analysis.

We found only one recent Australian study where the frequency of spermatozoa was analyzed from first void urine specimens.[1] They detected spermatozoa in 35% of first void urine specimens collected following alleged penile-vaginal penetration.[1] To our knowledge, there are no published studies analyzing the frequency

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of spermatozoa for a longer period than from the first void urine. Although DNA identification is used to obtain evidence in sexual assault, a Y-DNA analysis of urine has not been yet done for forensic purposes.

The purpose of the study was to evaluate the detection rate of Y-DNA in post-coital urine samples and to evaluate the time frame for positive Y-DNA following consensual intercourse and the detection rate of spermatozoa in urine samples.

2. Material and methods

2.1. Participant characteristics

Female volunteers, comprising medical or laboratory students, hospital personnel or acquaintances, were invited to participate in a research study collecting and analyzing urine samples following consensual vaginal intercourse from May 2008 to December 2009 by either a personal invitation or by collective invitation after a lecture. 90 volunteers took part in the study. Two volunteers were excluded because of misunderstandings in filling in the study information form. 88 volunteers were included in the study. Most of the study participants had participated also in other studies evaluating collection of forensic evidence.[2,3]

The women were instructed to record the time of their last intercourse before the studied urine collection, the urine sample collection time and which of the consecutive post-coital voidings (1st, 2nd etc.) was collected as a specimen. A medical history was given by the volunteer using a form. Contraception, time of previous intercourse before the intercourse for the sample collection, frequency of intercourse in the past two weeks prior to the sample collection, activities following the intercourse (wiping, washing, showering, going to the sauna, urinating, defecating) and duration of intercourse were recorded.

2.2. Sample collection

Following consensual intercourse, volunteers were advised to void into a collection bottle (volume 500 ml, Sarstedt prod. no 77.582, Germany) so that the rim of the collection bottle would lie tightly onto the perineum. The purpose was to collect the outflow of vaginal secretions as much as possible into the collection bottle when physiologically relaxing the pelvic floor muscles while urinating. The urine volume was collected as much as possible into the collection bottle. The volunteers were advised not to worry if the urine volume exceeded the volume of the collection bottle. The first post-coital voiding urine sample was instructed to be collected without washing or without any other possible post-coital activities. Post-coital activities were allowed for the following collected urine samples.

A single-use collection bottle was used for each episode of voiding. The urine collection bottle(s) were used for preserving the urine in the refrigerator. The collection bottle was labelled by (1) the study number given to the volunteer earlier during invitation and the volunteer (2) numbered the post-coital voiding time and marked it on the side of the collection bottle. The collection bottle was returned to the examiner as soon as possible but not later than 5 days.

Time of the studied intercourse, time of the collected urine sample, post-coital activities, and the numbering of post-coital voiding times were recorded on the study information form.

2.3. DNA extraction and quantification

The amount of urine and the pH of the urine were measured. The entire volume in the urine samples were centrifuged for 10 min at 3000 rpm and the supernatant was decanted. After centrifugation the supernatant (urine) was poured out and the cellular deposit at the base of the centrifuge tube was collected for analysis. From 200 μl of cell pellet DNA was extracted using QIAamp DNA Mini Kit® (Qiagen, Germany). Centrifugation steps were carried out at room temperature according to the protocol.

Quantifiler Y Human Male DNA Quantification Kit® (Applied Biosystems, USA) was used to quantify the total amount of amplifiable human male DNA. PCR reactions and analysis were done with AbiPrism® 7000 HT Sequence Detection System (AppliedBiosystems, USA) according to the instructions provided. The PCR of Quantifiler Y Human Male DNA Quantification Kit® method is standardized and we used recommended water as blank controls alongside the purification and amplification steps. A sample was considered positive if a measurable amount of DNA was detected. The cut-off limit for possible DNA identification of the male was considered to be 0.01 ng/μl and according to the recommended cut-off limit of 0.023 ng/μl for Quantifiler Y.

2.4. Microscopic analysis

Immediate microscopy was performed in a subset of 111 (54.1%) urine samples from 47 volunteers in a forensic laboratory. The following parameters were examined: volume, motility and sperm density. The density was categorized to no sperm, a few sperm (1–10/slide), a moderate amount (10–50/slide), and many (>50/slide). The examination was performed according to the WHO laboratory manual 1999.[4] Each sample was suspended on a slide by mixing the sample with a small amount of a culture medium (5 μl). The covered preparation was examined under the microscope. All samples were examined by phase-contrast microscope (magnification of 10 × 20).

2.5. Contamination issues

The possibility of contamination has to be considered. All the urine sample processing, extraction and amplification steps were performed by the same female experimenters to avoid secondary Y-DNA contamination. Disposable powder free gloves were used and changed frequently to minimize the contamination risk.

2.6. Ethical considerations

The study protocol was approved by the Ethics Committee (#R08018) of Pirkanmaa Hospital District, Finland. Written consent was required for participation in the study, for forensic laboratory analysis and for publication purposes. No data from the patient's medical records were collected. Full anonymity was secured in storage and laboratory analysis.

2.7. Statistical considerations

Results were summarised by descriptive statistics; medians with range or frequencies (n) and percentages (%). Differences between detection of Y-DNA and spermatozoa were analyzed using the McNemar test. The Chi-square test was used to compare our microscopy results on detected spermatozoa rate in first void urine to results from the study by Smith et al. (2014). SPSS 21 was used for data analysis (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).