Increased Male Evidence Detection Rates by Utilization of DNA Markers in Forensic Rape Cases of Espírito Santo, Brazil

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Abstract

Sexual assault ignores cultural barriers, social class, socioeconomic and personal limitations. Cases of rape, murder and other forms of sexual abuse have been recognized as a health problem. These cases have been targeted by forensic sciences, with the goal of developing more effective strategies for the identification and punishment of aggressors. Seminal biomarkers have already been used for detection of sperm in vaginal samples in cases of sexual violence, but in some cases biological fluids represent a mixture of serological types, making it impossible to identify the perpetrator. Since 1985, DNA has been used as a tool of genetic identification, bringing progress and helping solve many criminal and civil cases. In rape forensic investigations, the conclusive identification of semen is required to corroborate the alleged sexual crime. However, even when sperm cells are absent, various types of non-sperm cells are left by the perpetrator (epithelial cells, leukocytes, immature germ cells), representing viable sources of DNA for analysis. Aiming to identify the presence of male DNA in samples of women victims of sexual violence, we analyzed 132 sexual abuse samples. Using five specific molecular markers for the Y chromosome, it was possible to identify 19 positive samples, which were negative for SC and PSA. Primers AMEL Y, SRY D&E and DYS270 showed high score rates, sensitivity, specificity and accuracy, suggesting that this new method presents good reliability and gives new perspectives for the sample screening process, providing more information about forensic samples involving sexual crimes in the Espírito Santo State, Brazil.

ABBREVIATIONS

AMEL: Amelogenin; APA: acid phosphatase activity; APS: Amonium persulfate; Art.: article; AS: Autosomal; BP: base pairs; DNA: Desoxirribonucleic acid; dNTP: Desoxirribonucleotides triphosphate; EDTA: ethylenediaminetetraacetic acid; PCR: polymerase chain reaction; PSA: Prostate-specific antigen; SC: spermatoscopy; NGHM: Núcleo de Genética Humana e Molecular (Center of Human and Molecular Genetics); LoTE: tris/EDTA pH 8 buffer; SDS: sodium dodecyl sulfate; STS: Sequence-Tagged Site; Y-STR: Y Short Tandem Repeats; STR: Short Tandem Repeats; SRY: Sex-Determining Region of the Y chromosome; V: volts; μl: microliters.

INTRODUCTION

Investigation of rape cases in the forensic field requires a conclusive identification of semen to confirm an alleged sexual assault. Victim’s medical examination and laboratory analyses of biological samples aim to detect the perpetrator’s semen and supply legal evidences for the criminal process. Vaginal
swabs are, in these cases, the most reliable source of biological evidence, despite the enormous variation of samples condition and degradation degree.

Biochemical techniques most recommended for routine forensic rape analyzes include sperm cytology (SC), acid phosphatase activity (APA) and detection of prostate-specific antigen (PSA) [1]. SC is the gold standard confirmatory test; APA is a presumption test [2], whereas PSA detection represents a more specific marker [3]. The PSA test was introduced in criminal investigations of rape in 1971 [4] and it has been accepted as a confirmatory test in rape investigations since then, especially because detectable levels may persist in the vagina up to 48 hours after intercourse [5]. All these biomarkers have shown different stabilities in the vaginal fluid [5] which may cause misinterpretation of results.

Seminal markers have long been used in cases of sexual violence [6,7], however, in approximately two thirds of cases, it is not possible to identify the semen’s donor, because mixed biological fluids result in mixed serological types.

Even when sperm is absent, several types of non-sperm cells are left by the perpetrator (epithelial, leukocyte, immature germ cells) and represent a viable source of DNA for analysis. These cells are normally found in approximately 15% of sperm materials [8] provided by the prostate gland and seminal vesicles as major sources.

More recent methodologies based on detection of DNA (autosomal and Y-chromosome short tandem repeats [AS- and Y-STRs]) have been described for forensic purposes [9] and offer not only the potential benefit of higher sensitivity and specificity than biochemical ones but the possibility of assailant identification. STR detection to identify or exclude possible suspects brought a breakthrough in forensic cases around the world. Its utilization more than tripled from 2001 to 2010, with over 3500 publications detailing the technology and reporting allele frequencies for STR loci in hundreds of populations worldwide [10].

With the purpose to increase perpetrator DNA detection rates in rape cases, the present work aims to study the efficacy of PSA and SC tests, when compared to DNA based tests in forensic analysis of rape.

MATERIALS AND METHODS

PSA test and Sperm cytology

PSA and SC tests were performed by experts in the Toxictology Laboratory of the Civil Police of Espírito Santo State. PSA scores were determined by immunochromatographic membrane test assay performed by One Step ABA card (Imuno-Rapido PSA commercial kit [WAMA Diagnostica Ltda, SP, Brazil]) according to manufacturer’s recommendations. SC test was performed by experienced professionals using the smear slide technic and Corin & Stockis coloration method [11]. Preparations were microscopically screened for spermatozoa under 100x magnification and the total cell number was scored. The sample was considered positive when at least 1 sperm (usually head) was unequivocally observed.

Vaginal swabs

Samples were provided by the Toxictology Laboratory of the ES Civil Police, consisting of 80-100µL of the first swab wash, after tested by routine PSA and SC, at which point samples would be discarded. Vaginal swabs were collected using cotton swabs, by forensic examiners at the Police Legal Medicine Department in ES State, Brazil, during March 2012- February 2013. For DNA evaluation studies, 132 samples were tested. Samples were stored at -20°C until analyzed.

DNA extraction and evaluation

Samples from 132 casework swabs were subjected to Proteinase K and SDS treatment followed by organic phenol/chloroform extraction and ethanol precipitation [12]. DNA from each sample was extracted and resuspended in 30 µl of LoTE buffer, 5µl was used per reaction. The Y chromosome regions analyzed in this study were amplified by PCR (Mastercycler Personal Eppendorf) in simplex reactions, using primers described by Fuqua et al. 1997 [13] and modified primers described by Carvalho et al., 2007[14] (table 1).

Reactions were performed using a male DNA positive control (mandatory amplification) and a female DNA negative control at dilutions of 1:100. Contamination controls were represented by ultrapure water pipetted simultaneously with the test samples divided into: (1) laminar flow pipetting (pre-PCR control) and (2) bench pipetting (DNA control). In both cases, no amplification was expected.

After amplification, 6µl of PCR product were applied to a 7% polyacrylamide gel with a 50bp molecular weight marker, run at 240V for 60 to 90 minutes, silver nitrate stained according to Sanguinetti et al. 1994 [15] and photo documented.

Results interpretation, sensitivity and specificity

Amplification of specific regions of the Y chromosome detects the presence of male DNA in the swab wash. Positive scores were considered when three or more markers showed positive after PCR. Presence of male DNA was compared with results from SC (gold standard test) and PSA tests.

Analysis of sensitivity and specificity were performed to validate the methodology [16].

RESULTS AND DISCUSSION

Male DNA detection by PCR

When compared with sperm cytological analysis, PCR amplification detected 14 out of 17 positive samples, corresponding to a sensitivity of 82%. This difference can be justified by factors such as the improper handling and storage of samples, which may result in degradation of male genetic material by human or bacterial nucleases [17]. Cytological analysis errors may also have influenced results [18].

Among 115 cytologically negative samples for spermatozoa, 29 were positive for male DNA, of which 10 also showed positive in the PSA test, increasing the reliability of DNA results.

As for PSA, of 43 positive samples, 24 showed positive by PCR (56% sensitivity). The lower sensitivity may be explained by the
fact PSA is a protein based test, which may be positive even in the absence of male cells.

We attributed particular importance to the 19 positive samples by PCR which were among the 89 negative samples for PSA. This represents a 20% fraction of false negatives by PSA analysis and strongly supports the use of DNA based tests in forensic rape case work, highlighting the importance of affordable and simple genetic testing in Police routine cases of sexual violence.

Sensitivity and specificity of DNA based tests, showed satisfactory results, presenting values higher than 75% of concordance with cytological results (gold standard). When markers were evaluated separately, sensitivity ranged from 64% (AMEL Y) to 76% (SRY F & G). We considered specificity satisfactory for markers AMEL Y, SRY D & E and DYS270 (above 75%), and amelogenin (89%).

As Sartori in 2008 [19], best results in detecting portions of the Y chromosome in low representation mixtures was using primers SRY D & E and AMEL Y, which we recommend as additional tests for forensic rape cases, whereas marker DYS270 was also satisfactory.

CONCLUSION

Our results show that the sole utilization of cytological and biochemical markers (SC and PSA) is not sufficient for robust detection of male evidence in female rape cases, and therefore an association with simple and affordable molecular techniques is highly recommended. By doing so we were able to increase by 20% PSA male detection rates and confirm a sexual crime. Furthermore, we suggest that a further analysis with autosomal and Y-STR markers could allow for the identification of perpetrator’s specific STR alleles [20]. Markers AMEL Y, SRY D&E and DYS270 showed the best results overall, being considered reliable to be used in male DNA identification. In conclusion, we recommend the combination of DNA based tests and regularly used PSA and SC tests to increase male evidence detection rates in forensic rape case work [21].

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REFERENCES


