ABO genotyping of the suspects using their sperm DNA.

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Introduction

In sexual assaults against women, the key to identify the suspect is ABO phenotyping or the typing of other polymorphic markers of the seminal fluid in the victim's vagina. However, ABO phenotyping is frequently unsuccessful, since mixtures of fluids cannot be separated to be subjected to conventional methods for the detection of antibody or antigen material. We therefore studied ABO blood group genotyping of isolated sperm DNA from contaminated vaginal fluid by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [1].

Materials and Methods

10 μ l of post-coital vaginal fluid in 4 sexual assaults were collected from the victim's vaginae with syringes within 24 hr after the crimes. We have examined the two-step extraction procedure for purification of sperm DNA or vaginal epithelial DNA in contaminated specimens by a modification of the NaI method [2,3]. The first step of digestion was to solubilize the non-sperm cells in the mixed fluids. Since sperm nuclei are protected by cross-linked thiol-rich proteins, they remained intact in the first step of digestion without DTT[3]. Thus, the mixed fluids were separated into the sperm heads and the female components by centrifugation. In the second step of digestion, the precipitated sperm heads were solubilized with DTT. And these DNA were then subjected to the PCR-RFLP with a restriction enzyme, Kpn-1 or Msp-1, for each site [1,3]. All specimens were genotyped as shown in Table 1.

ABO genotype	Primer 1f and 1r Kpn-1	Primer 3f and 3r Msp-1
OO	69	140
AA	96	140
AO	96,69	140
BB	96	159
BO	96,69	159,140
AB	96	159,140

(bp)

.e. 1 ABO genotyping by PCR-RFLP

Results

In case 1, only the 69 bp DNA fragment of locus 1 and the 140 bp DNA fragment of locus 3 were found in the digested PCR products of the recovered sperm DNA (lane 2 of Fig 1a, b), whereas the 96 bp DNA fragment of locus 1 and the 140 bp and 159 bp DNA fragments of locus 3 were found in the digested PCR products of the recovered vaginal cell DNA (lane 3 of Fig 1a, b). These findings clearly showed that the genotype of the male suspect was OO and the genotype of the female victim was AB (Table 2). Since the band of the 96 bp DNA fragment of locus 1 and the 159 bp DNA fragment of locus 3 were not found in lane 2 shown in Fig 1a, b. These findings show that sperm DNA extracted by these methods was not contaminated by any female components.

In case 2, the genotype of the male suspect was OO and the genotype of the female victim was BO (Table 2, lane 6 of Fig 1a, b). There was no band of the 96 bp DNA fragment of locus 1 or the 159 bp DNA fragment of locus 3 in the recovered sperm DNA, demonstrating that the sperm DNA did not contain any female components.

In cases 3 and 4, the genotype of the male suspect was AB and the genotype of the female victim was AO (Table 2, lane 6 of Fig 1a, b). There were no bands of the 69 bp DNA fragment of locus 1 in the recovered sperm DNA. These findings indicate that the sperm DNA extracted by these methods did not contain any female components.

Discussion

With this method, mixed samples can be completely separated into the male and female components, and the genotype of the recovered sperm DNA or vaginal epithelial cells can easily be determined. This reliable ABO genotyping method by PCR-RFLP, using separated sperm DNA, should be of value in forensic identification in sexual assaults [3].

References

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2. Ishizawa, M., Kobayashi, Y., Miyamura, T. and Matuura, S. Simple procedure of DNA isolation from human serum. Nucleic Acids Research, Vol. 20, 1991, p 5792.

3. Sasaki, M and Shiono, H. ABO genotyping of suspects from sperm DNA isolated from the post-coital samples of sexual crimes. J.Forensic Sci.1996, 41 (3) submitted.

Case	ABO phenotypes of semen-contaminated vaginal fluid	ABO phenotypes of victim	Possible ABO phenotypes of suspect	ABO genotypes of recovered sperm-DNA by PCR-RFLP
1	AB	AB	AB, A, B, O	OO
2	B	B	B, O	OO
3	AB	A	AB, B	AB
4	AB	A	AB, B	AB

Table 2 ABO genotypes of of recovered sperm-DNA by PCR-RFLP from semen-contaminated vaginal fluid in 4 sexual assaults.

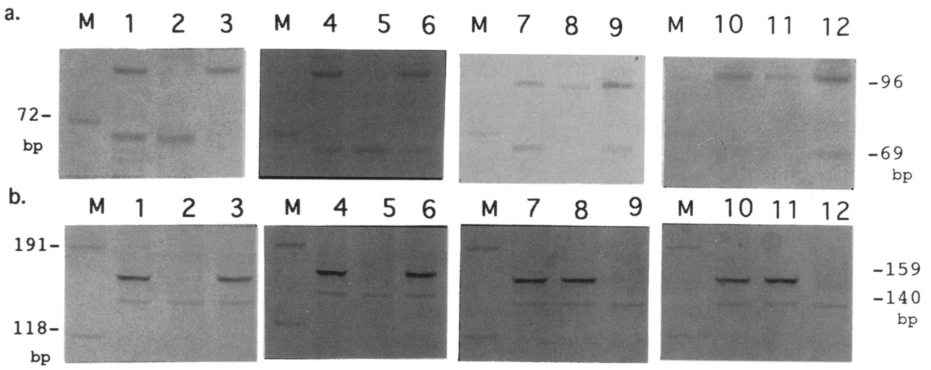

Fig. 1 - Electrophoresis in 10% polyacrylamide gels of each digested PCR product. a; PCR products of Locus 1 digested with Kpn-1. b; PCR products of Locus 3 digested with Msp-1. M: ϕX174/Hae III digest, Lane 1-3; Case 1, Lane 4-6; Case 2, Lane 7-9; Case 3, Lane 10-12; Case 4. Lane 1, 4, 7, 10; contaminated vaginal fluid, Lane 2, 5, 8, 11; recovered sperm DNAs, Lane 3, 6, 9, 12; recovered vaginal epithelial DNAs.