TECHNICAL NOTE

Akira Harada,[1] *B.S.; Kazuo Umetsu,*[1] *M.D.; Isao Yuasa,*[2] *M.D.; Noriaki Ikeda,*[1] *M.D.; and Tsuneo Suzuki,*[1] *M.D.*

Detection of Orosomucoid 1 Phenotypes in Semen and Semen Stains

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ABSTRACT: Orosomucoid 1 phenotypes were detected in seminal plasma by isoelectric focusing and immunoprinting. The orosomucoid 1 phenotypes in seminal plasma correlated with the types found in the corresponding serum specimens. Semen stains stored for ten days could be typed for orosomucoid 1. The present work revealed that orosomucoid 1 is a useful genetic marker for the medicolegal grouping of semen stains.

KEYWORDS: forensic science, genetic typing, semen, orosomucoid type, orosomucoid 1, semen stains

It has been pointed out that several genetically variable protein, enzyme, and blood group markers that offer great potential for genetic typing are contained in semen. However, there are a number of problems that must be overcome before genetic typing of semen stains can become of practical use, and only a few of the genetic markers, such as ABO, phosphoglucomutase, and Gm, Km systems, have been used in forensic science casework [*1-3*]. It is desirable that a practical new method for genetic typing analysis of semen stains is established.

Orosomucoid (ORM, α_1-acid glycoprotein) is normally present in human serum at concentrations of 0.5 to 1.0 mg/mL and is known as an acute-phase reactant. The ORM polymorphism has been revealed by Tokita and Schmid [*4*], Johnson et al. [*5*], and Umetsu et al. [*6*]. ORM is encoded at two structural gene loci, ORM1 and ORM2, and ORM1 is genetically polymorphic with two common alleles, *ORM1*1* and *ORM1*2* [*7*]. Recently a duplicated allele, *ORM1*2 · 1*, has been described [*8*]; this allele is common in Japanese and Taiwanese populations [*8, 9*] and has been observed in Caucasians [*10*]. ORM is present in semen [*11*]. We demonstrate here that the polymorphic ORM1 types can be detected in semen and semen stains.

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[1]Forensic scientist, forensic scientist, forensic scientist, and professor, respectively, Department of Forensic Medicine, Yamagata University School of Medicine, Yamagata, Japan.

[2]Forensic scientist, Department of Legal Medicine, Tottori University School of Medicine, Yonago, Japan.

Materials and Methods

Serum and semen specimens were collected from 27 healthy volunteers. The seminal plasma specimens were obtained from semen following high speed centrifugation (10 000 rpm, 1 min). The seminal plasma and the serum specimens were stored at −30°C until analyzed.

The concentrations of the ORM in the serum and the seminal plasma specimens were measured by the single radial immunodiffusion (SRID) technique. Immunodiffusion was carried out in 1% agarose gel containing veronal buffer (μ = 0.025, pH 8.6), and 1 and 0.5% anti-ORM immunoglobulin (Dakopatts, Denmark) for serum and semen, respectively. The wells (2.5-mm diameter) punched in the gel were filled with accurately measured volumes of the seminal plasma and serum as well as reference solution with known concentrations of antigen (QS serum, Hoechst Japan, Tokyo). The plates were incubated in a humid box at room temperature for 48 h and then treated with 1% tannic acid solution to increase the sensitivity [*12*]. The precipitate rings were measured directly. Standard calibration curves for serum and seminal plasma were separately established by using serial dilutions of reference antigen solution, and the concentrations of antigen in serum and seminal plasma specimens were determined by comparison to the respective standard curves.

One microlitre of serum was treated with 80 μL of 0.2*M* sodium acetate buffer, pH 5.0, containing 1 U/mL neuraminidase from *Clostridium perfringens* (Type V, Sigma, St. Louis, MO) and left overnight at room temperature. Ten microlitres of seminal plasma specimens were treated with 30 μL of the neuraminidase solution.

Polyacrylamide gels (5% T, 3% C, 110 by 120 by 0.5 mm) were prepared containing 2.4% w/v carrier ampholyte, 12% v/v glycerol, and 0.2% v/v Triton X-100 [*13*]. The pH range of carrier ampholyte used in the present experiment was pH 4 to 6.5 (Pharmacia). The catholyte and anolyte were 0.2*M* sodium hydroxide (NaOH) and 0.5*M* phosphoric acid (H_3PO_4), respectively. Serum and seminal plasma specimens were applied on filter papers (4 by 15 mm, Whatman No. 3). They were placed at a distance of about 1.5 cm from the cathode. The gel was prerun for 30 min at 10°C and at a constant power supply of 5 W. Once specimens had been applied, focusing was continued for 1.5 h at a constant power supply of 10 W. The filter papers were removed after 30 min of focusing. An immunoprinting after isoelectric focusing (IEF) was performed using a cellulose acetate membrane (Separax, Fuji Film, Tokyo) soaked in a 1/4 dilution of anti-ORM immunoglobulin (Dakopatts, Denmark). The contact time was 1.5 h. The removed strips were washed for 30 min with 0.01*M* phosphate-buffered saline (pH 7.2) containing 0.01% v/v Tween 20, and they were stained with Coomassie Brilliant Blue R-250.

Semen stains were made on 4 by 6-mm filter paper (Whatman No. 3), and they were allowed to dry at room temperature. The semen-stained filter papers were soaked in 20 μL of the neuraminidase solution overnight at room temperature in a humid box and applied onto the gel surface directly. The IEF and immunoprinting procedure were the same as for serum and seminal plasma specimens.

Results and Discussion

The ORM could be detected in all seminal plasma specimens. The concentration ranges of the ORM in seminal plasma specimens were 0.007 to 0.145 mg/mL (mean 0.03 mg/mL). The result coincided approximately with that reported by Lizana and Blad [*14*]. Figure 1 shows the correlation of the ORM concentrations between serum and seminal plasma as measured by the SRID assay. The result revealed that the ORM concentrations in seminal plasma are 20 to 1.2% (mean 4.1%) of those in serum.

ORM1 phenotyping of serum, seminal plasma, and semen stains is shown in Fig. 2. Five phenotypes were observed in 27 specimens: ORM1 1 (15), 2-1 (4), 2 (1), 2 · 1-1 (6), and

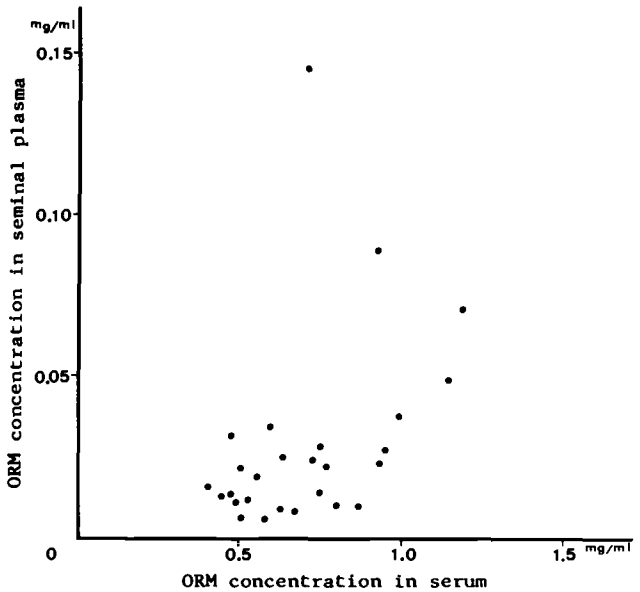

FIG. 1—*Correlation of ORM concentration between serum and seminal plasma (*n = *27,* r = *0.4048).*

2 · 1-2 (1). Phenotypes expressing the duplicated allele *(ORM1*2 · 1)* are recognized by a difference in band intensities [*8*]. ORM1 phenotypes with this allele must be carefully determined. It is true that the differences of band intensities might pose a typing problem, for example, a weakly staining ORM1 2 · 1-1 might type as a ORM1 1. The intensity of the ORM1 1 band and that of the ORM1 2 band is about 3:1. Even in the seminal plasma specimens with the lowest ORM levels, ORM1 2 · 1-1 and ORM1 2 · 1-2 could be correctly phenotyped by the present method. Thus, the ORM1 types in seminal plasma specimens correlated with those found in the corresponding serum specimens, and there were no examples of noncorrelation. There was not any difference in the expression of the ORM1 locus in the subjects with any seminal ORM levels. In ORM patterns of seminal plasma specimens, the extra bands that were not detected in those of serum specimens appeared frequently, but ORM1 typing was not disturbed by the bands (Fig. 2*a*,*c*: ◁). They seemed to be some products of an additional ORM locus. When the seminal ORM concentrations were too high, the ORM typing was disturbed by the tailing of the band pattern. In these seminal plasma specimens, the clear band patterns could be obtained by reducing the specimen volume applied on the filter papers.

In the present work we did not deal with ORM2 typing because the ORM2 type of most Japanese is ORM2 1.

All semen stains (stored ten days) could be ORM1 typed correctly and clearly by the IEF immunoprinting technique (Fig. 2*c*), and the types of the semen stains coincided with those of the corresponding serum specimens.

The polymorphism of ORM1 was confirmed in seminal stains, and the present work revealed that ORM1 is a useful genetic marker for medicolegal grouping of semen stains. The method is simple, fast, and easy to perform. Owing to the appearance of the extra bands in ORM1 typing from semen stains, however, control specimens from serum are required in trying to use this polymorphic system in any forensic science casework.

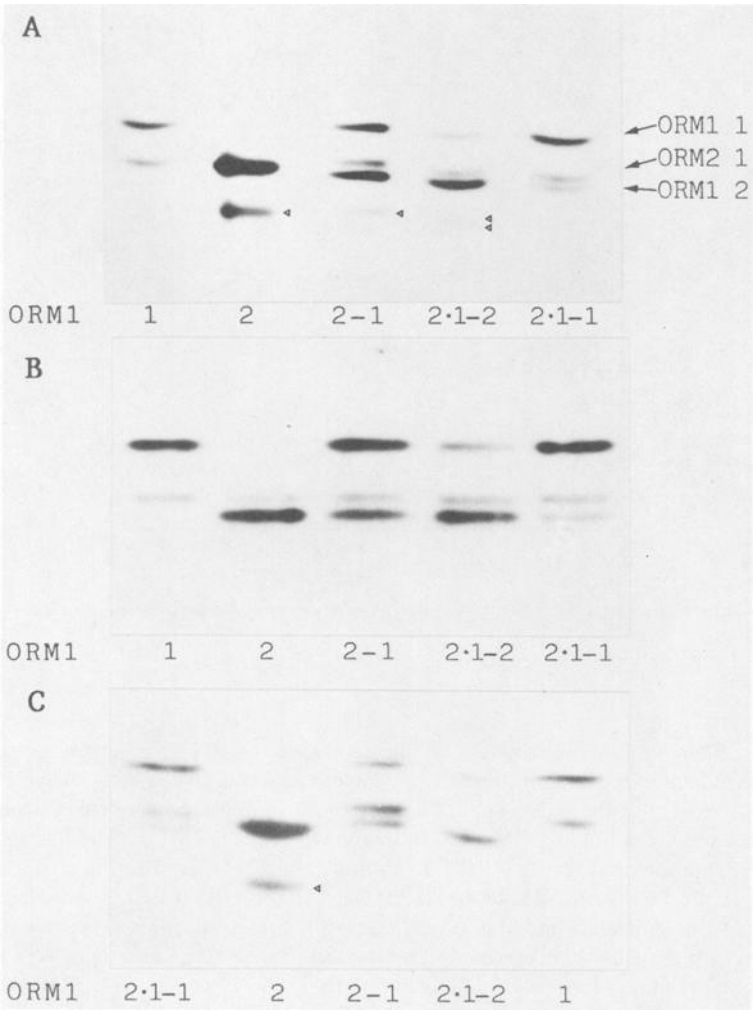

FIG. 2—*ORM1 types represented by immunoprinting after IEF. Semen stains were stored at room temperature for ten days. Anode at the top.* (a) *seminal plasma,* (b) *serum,* (c) *semen stains, and* ◁*: extra band.*

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Address requests for reprints or additional information to
Tsuneo Suzuki, M.D.
Department of Forensic Medicine
Yamagata University School of Medicine
Yamagata, 990-23 Japan