Improved Diagnosis of Orosomucoid (ORM) Phenotypes by isoelectric focusing in immobilized pH gradients. Comparison with other phenotyping methods

A. Carracedo, M.D. Montiel, M.S. Rodriguez-Calvo, L. Concheiro, E. Huguet(*) and M.Gené(*)

Dep. of Legal Medicine. University of Santiago de Compostela (Spain) and (*) Dep. of Legal Medicine. University of Barcelona (Spain).

INTRODUCTION

Alpha-1-acid glycoprotein or orosomucoid (ORM) is a globulin characterized by a very high carbohydrate content (45%), a large number of syalyc residues and an extremely acidic isoelectric point (pI 2.7-3.5)(Schmid 1975).

Although ORM polymorphism was demonstrated twenty years ago (Schmid et al. 1965), it is not habitually used in paternity testing and bloodstain analysis due to the technical difficulties involved in the methods used until recently.

In the last few years, because of the utility of this protein in forensic haemogenetics, ORM polymorphism has been investigated using modern methods. Umetsu et al.(1985), Thymann and Eiberg (1985) and Carracedo et al. (1985) have proposed different methods for ORM analysis, which permit a reliable characterization of ORM phenotypes.

In this paper we present the results of ORM typing by several methods, including isoelectric focusing (IEF) in acidic pH ranges followed by silver staining, print immunofixation, fixation with a lectin from the sea-weed "Fucus tomentosum", IEF followed by immunofixation in miniaturized gels and IEF in immobilized pH gradients.

MATERIALS AND METHODS

Freshly collected blood samples from healthy adults proportionally representative of Galician regional districts were used. Blood samples were collected by syringe into 5 mL tubes containing heparin as anti-coagulant. Plasma was removed and stored at -20°C. Samples were processed within a year after extraction.

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

IEF in acidic pH ranges followed by silver staining

Polyacrylamide gel isoelectric focusing was carried out in 0.4 mm polyacrylamide gels at a gel concentration of T=5% and C=3%. A mixture (1:1) of Pharmalyte 2.5-5 and Ampholine pH 2.5-4 was used in order to obtain an appropriate pH range. Ampholyte concentration was 2.8%. Samples were applied with Whatman 3 filter papers at a distance of 2 cm from the cathode. Isoelectric focusing was carried out at 5 W for 3 h. After focusing the gels were stained with the silver staining method of Carracedo et al. (1983).

IEF of desialyzed serum followed by immunofixation and print lectinofixation

A mixture (1:1) of Ampholine pH 4-6 and Pharmalyte pH 4-6.5 was used. Desialylation of sample was carried out by incubation of 5 µl serum with 20 µl neuraminidase (1U/ml) at 37°C for about 24 h prior to IEF. Samples were applied at a distance of 2 cm from the cathode using Whatman paper. Electric focusing was carried out at 5 W for 3 h.

After focusing immunoprinting was carried out with anti-ORM from Atlantic Antibodies which was diluted 1:1 and applied directly onto the surface of the gel. After 30 min of incubation at 37°C the gels were washed for 24 h with saline and then they were stained with Coomassie R-250. ORM patterns were also obtained by print lectinofixation (time of contact 30 min at 37°C) using a cellulose acetate strip soaked in 0.1mg/mL of a lectin which was prepared by affinity chromatography described previously (Muñoz-Crego 1987). The removed strips were washed for 2h with saline and then stained with Acid Violet 49.

Alternatively miniaturized gels were used, runned with the new PhastSystem (Pharmacia, Uppsala, Sweden). A pH range of 4-6.5 was used. The runs takes 500 Vh or approximately 25 min. Immunofixation was then carried out as described above but the incubation takes only 10 min and the washing step 4 h.

IEF in immobilized pH gradients

Ultrathin layers IPGs were performed as described previously in the LKB application note n.324 (1984). Samples of desialyzed serum were first tested on broad IPG pH 4-5 (buffering/titrating amounts as in the same LKB note). Twofold or more deeper intervals were then derived from such gradient by simple linear interpolation. The pH interval of 4.2-4.8 was particulary useful.

Immunofixation and print lectinofixation were carried out as described above.

RESULTS AND DISCUSSION

Fig. 1 shows the ORM pattern after isoelectric focusing in acidic pH ranges. A clear distinction between phenotypes was found and the silver staining render the use of immunotechniques unnecessary.

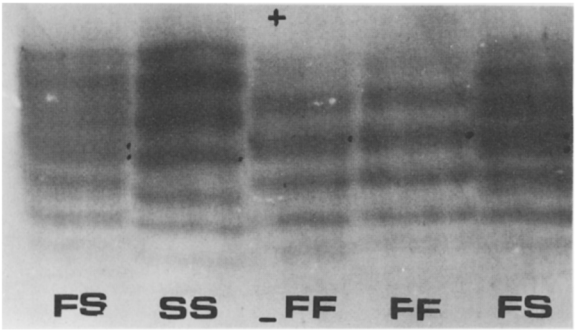

Fig.1 ORM phenotypes by IEF in the pH range 2.5-5 followed by silver staining.

Fig.2 shows the ORM pattern after IEF followed by print immunofixation. The ORM band pattern with the lectinofixation and those with the immunofixation are much the same. Phenotypes can be clearly distinguished but a better result is obtained including separators (MES and ACES) as described previously Thymann and Eiberg(1985).

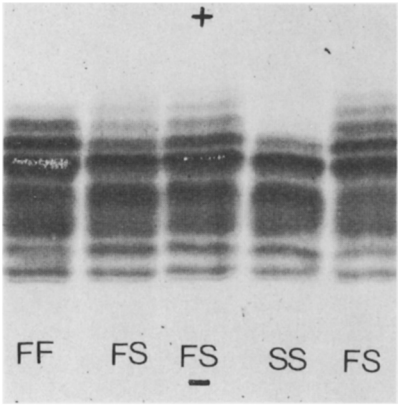

Fig.2 ORM pattern of desialyzed serum after IEF in the pH range 4-6.5 followed by immunofixation

The lectin obtained from the sead weed "fucus tomentosum", reacts with various serum protein including ORM. Other lectin obtained from the beetle "Allomyrina dichotoma", was recently used for the detection of ORM phenotypes (Umetsu et al. 1985). This suggested that some lectins would contribute to the demonstration of polymorphism of some other serum group systems by print lectinofixation after IEF.

The best resolution was achieved using ultranarrow immobilized pH gradients. Furthermore rare additional ORM S subtypes were found, (Fig.3), and although only a few family studies were carried out until now, the codominant inheritance of this allele seems to be clear.

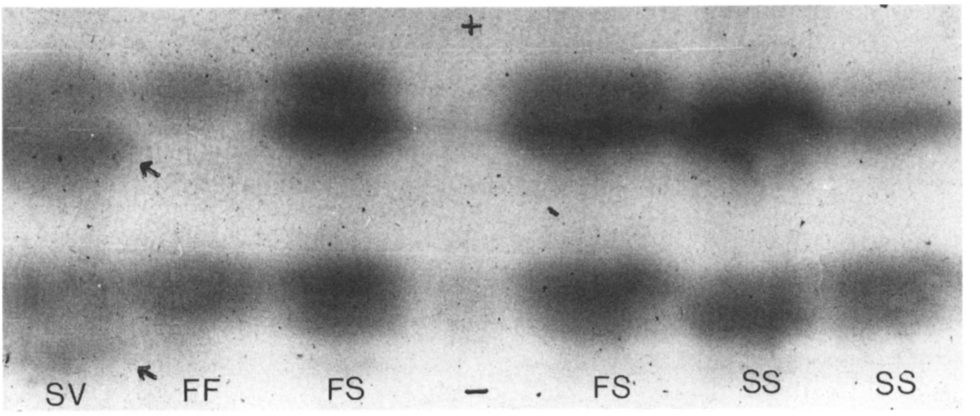

Fig.3 ORM patterns on a shallow immobilized pH gradient (interval of pH 4.2-4.8).

The use of miniaturized gels with the PhastSystem gives also very good results. The adventages of this method are the resolution, reproducibility and the economy. Furthermore the gels can be stored and projected like slides.

REFERENCES

Carracedo A, Concheiro L, Requena I, Lopez-Rivadulla M (1983) A silver staining method for the detection of polymorphic proteins in minute bloodstains after IEF. For Sci Int 23:241-248

Carracedo A, Concheiro L, Estefania J, Suarez C (1985) Polymorphism of serum orosomucoid. Family and population studies in Galicia. Advances in Forensic Haemogenetics I. Springer-Verlag, Berlin, p 282

Muñoz-Crego, MA (1987) Doctoral Thesis. Univ. Santiago de Compostela

Schmid K, Tokita K, Yooshizaki H (1965) The alpha-1-acid glycoprotein variants of normal caucasian and japanese individuals. J Clin Inves 44:1394-1401

Schmid K (1975) Alpha-1-acid glycoprotein. In Putnam FW (ed) The plasma proteins, vol I. Academic Press, New York p 183

Thymann M, Eiberg H (1985) Orosomucoid polymorphism: Determination by separator isoelectric focusing and demonstration of ORM F subtypes. In Brinkmann B, Henningsen H (eds.) Advances in Forensic Haemogenetics 1. Springer-Verlag, Berlin p 162

Umetsu K , Ikeda N , Kashimura S, Suzuki T (1985) Orosomucoid (ORM) typing by print lectinofixation: a new technique for isoelectric focusing. Two common alleles in Japan. Hum Genet 71:223-224

ACKNOWLEDGMENT

This paper was supported in part by grant no. 2654/83 from the Comisión Asesora de Investigación Científica y Tecnica (Ministerio de Educacion y Ciencia)