SERUM PROTEIN DETECTION IN OLD DENTAL PULP

López-Abadía I, Martinez MA, Gremo A, R Cuesta JM

Dept. of Legal Medicine. University Complutense Madrid Spain

INTRODUCTION

Dental pulp has proven to be a very good source of biological evidence for forensic casework. Its location in a especially protected cavity makes dental pulp very valuable. When environmental conditions are unfavourable we can still find well preserved proteins in this location.

We investigated some genetic polymorphisms associated with serum proteins in dental pulp from teeth stored at room temperature for as long as five years. We tried to find out which proteins were more resistant to unfavourable envinronmental conditions.

MATERIAL AND METHOD

We used isoelectric focusing methods to obtain phenotypes for the following proteins: Gc protein (Gc), alpha-1-antitrypsin (Pi), transferrin (Tf), alpha-2-glycoprotein (α–2 -HS) and orosomucoid (ORM).

We tried different solutions for extraction (urea, neuraminidase, distilled water) and incubation time varied from 12 to 48 hours at 4°C. Isoelectric focusing was followed by immunofixation and Coomassie Blue staining or silver staining. For Tf, α-2-HS and ORM we used the PhastSystem (Pharmacia) separation and development unit but for Gc and Pi we used conventional isoelectric focusing methods.

Isoelectric focusing conditions for the PhastSystem:

ORM and α-2-HS

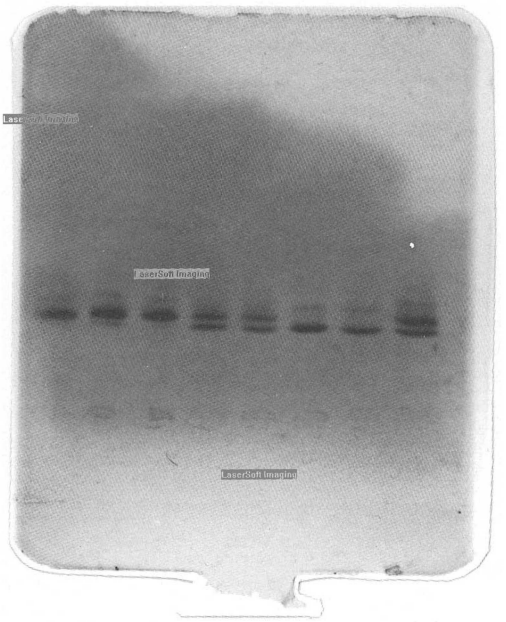
Sep 1.1	2000 v	10.0 mA	2.0 w	15°C	75 vh
Sep 1.2	2000 v	5.0 mA	1.5 w	15°C	20 vh
Sep 1.3	2000 v	25.0 mA	4.0 w	15°C	600 vh
Transferrin					
Sep 2.1	2000 v	2.0 mA	3.5 w	15°C	75 vh
Sep 2.2	200 v	2.0 mA	3.5 w	15°C	20 vh
Sep 2.3	2000 v	2.0 mA	3.5 w	15°C	700 vh

RESULTS AND DISCUSSION

We obtained clear reading of phenotypes when using recent pulps, but when teeth were very old or contaminated we could not determine phenotypes for Pi or Gc. Tf, α-2-HS and ORM showed more resistant and in most cases could be phenotyped using a minimum amount of sample (2 microlitres of eluate) even in the oldest specimens. Neuraminidase was the best extracting solution for these three serum proteins. A simple Coomassie Blue staining was used to determine phenotypes for Tf and α-2-HS. A silver staining showed more suitable for ORM in old dental pulps.

We were able to phenotype Tf, ORM and α-2-HS using the PhastSystem (Pharmacia) reducing considerable the time for assay.

We can therefore determine serum proteins such as the three mentioned above from very old dental pulp. This is especially useful when we have a very small amount of biological material because we use the same extracting solution (neuraminidase) and a minimum quantity of eluate.



Alpha-2-glycoprotein phenotypes in dental pulp.
From left to right: 1-1/1-1/1-1/2-1/2-1/2-2/2-2/2-1.

REFERENCES

Yuasa I, Umetsu K. *Genetic polymorphism of human alpha-2-HS-glycoprotein: Characterization and application to forensic hemogenetics.* Electrophoresis 1988; 9:404-410.

Katz and Cattone. *The present direction of research in forensic odontology.* JFS 1988; 33 (6):1319-1327.

Lee H et al. *Bits and pieces: serology, criminalistics, anthropology and odontology.* JFSS 1991; 31(2): 293-296.