

Determination of ESD Isozymes in Human Blood, Semen and Vaginal Secretion: Allelic Frequencies in Delhi Population

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ABSTRACT Isoenzyme band patterns of esterase D have been studied in blood, vaginal swabs free from semen, seminal fluid and their stains. ESD polymorphism was demonstrated in all the body fluids tested. One hundred and sixty two blood samples were analysed using starch agarose gel electrophoresis technique and the gene frequencies were estimated as $ESD^*1 = 0.765$ and $ESD^*2 = 0.235$. Stability studies carried out under different climatic conditions have revealed that the genetic marker is more stable in blood stains than in stains of semen and vaginal secretion.

INTRODUCTION

The ESD polymorphism is one of the most useful genetic markers in human genetics and forensic science. It is a member of the carboxylic ester hydrolase family ubiquitously distributed in the cells of many human tissues. ESD isozymes are distinguishable from other widespread esterases by their electrophoretic mobility and their substrate specificity for 4-methylumbelliferyl esters (Hopkinson et al. 1973; Coates et al., 1975). Three common phenotypes, ESD 1, ESD 2 and ESD 2-1 have been identified and are determined by two autosomal codominant alleles, ESD^*1 and ESD^*2 . There are also some rare genetic variants of the ESD (Marks et al., 1977; Sparkes et al., 1979; Koziol and Stepien, 1980; Olaisen et al., 1981; Nisugaki and Itoh, 1984).

The ESD gene has been assigned to the chromosome 13 q 14.11 region (Yunis and Ramsay, 1978; Sparkes et al., 1980) where the gene for susceptibility to retinoblastoma has been mapped.

Recently, Tsuchida et al. (1994) have analysed the esterase D polymorphism at the nucleic acid level. Two common alleles ESD^*1

and ESD^*2 were characterized by the substitution of one amino acid (gly-to-glu), which was caused by the point mutation of one nucleotide (G- to -A). Individual exhibiting the ESD1 and ESD 2 phenotypes were homozygotes for ESD1 and ESD2 DNAs respectively. Individuals sharing the ESD phenotype were found to have two kinds of DNAs, viz. ESD^*1 and ESD^*2 . The point mutation difference between the DNAs of the ESD^*1 and ESD^*2 alleles results in a different Ssp I digestion site. A restriction fragment length polymorphism caused by this difference with respect to Ssp I digestion site makes it possible to determine the ESD phenotype using DNA samples extracted from old forensic samples that have no detectable ESD activity.

Although the discovery of this enzyme in blood was made as early as in 1973, yet no clear picture emerged so far as its presence in body fluids viz. semen and vaginal secretions is concerned. One study reports its presence in semen (Blake and Sensabaugh, 1978) whereas others refute (Oepen et al., 1980; Sato et al., 1983). Likewise ESD variants could not be determined in vaginal secretions by Schwerd and Stock (Schwerd and Stock, 1982) but the results of later studies (Sato et al., 1983; Garlo, 1985) established its presence in vaginal secretions. In the light of contradictory reports, attempt was made to study ESD polymorphs in semen, vaginal secretion and their stains. Stability studies were carried out under different climatic conditions.

MATERIALS AND METHODS

Blood samples were collected from the blood bank of Indian Red Cross Society, New Delhi. The stains were prepared on cotton cloth pieces and allowed to dry in shade at room temperature.

Semen specimens were collected from volunteers and the stains were prepared in the laboratory. All the donors were healthy in the age group of 19-25 years. Sterilized cotton swabs were used to collect vaginal fluid from donors in the age group of 20 - 40 years and were ensured to be free from semen and blood. The time period since last intercourse varied from days to a few weeks when the donors supplied the samples. Six student donors in the age group of 19-22 who never had sexual intercourse also provided samples for the studies.

Haemolysates were prepared from cells thrice washed in normal saline, by freezing and thawing. Equal volumes of fresh lysates and 0.05 M Cleland's reagent (dithiothreitol) were taken in a cavity tile. The contents were mixed and kept in a moist chamber for 15 - 20 minutes at room temperature before application. The same treatment was given to semen specimens. Pretreated blood samples were absorbed on to Whatman-3 strips (0.8m × 0.1cm) and applied in the gel at about 2.5 cm from cathodic end. Similarly, pretreated liquid semen samples were directly applied into origin slits already made in the gel as previously reported (Sharma et al., 1988).

Dried stains of blood, semen and vaginal fluid were extracted with 0.05 M Cleland's reagent in a precipitin tube. 0.5 cm square of blood semen stained cloth piece and one third of vaginal stains (size varied depending upon the quality of stain) was cut and taken into a tube. To this was added a drop of Cleland's reagent and kept for 15 - 20 minutes at 4°C. Thereafter, tubes were centrifuged at 6000 rpm for 10 -15 minutes. Blood stain extract thus obtained was absorbed on Whatman 3 strips and applied in the gel. The sediment and supernatant fractions obtained from semen stains and vaginal swabs were mixed with a needle. The concentrated extract thus prepared was applied into origin slits already made in gel and run for electrophoresis.

The technique of Wrxall and Stolorow (1978) was standardised for analysing the samples.

RESULTS AND DISCUSSION

Table 1 shows the results of esterase D poly-

Table 1: Distribution of ESD Phenotypes in a heterogeneous Delhi Population

Phenotype	Observed number	Expected number	Allele frequency
ESD 1	98	95	$ESD^*1 = 0.765$
ESD 2-1	52	58	$ESD^*2 = 0.235$
ESD 2	12	09	
Total	162	162	

$\chi^2 = 1.82, 0.20 > p > 0.10$ for 1 d.f.; ns

morphism in blood samples drawn from heterogeneous population of Delhi. The study revealed that ESD 1 phenotype was most preponderant followed by ESD 2-1 and ESD 2. The allelic frequencies for ESD^*1 and ESD^*2 alleles were 0.765 and 0.235, respectively. The observed number of phenotypes were in good agreement with the number expected under Hardy-Weinberg equilibrium. Further, it was also observed that the occurrence of ESD^*2 gene among Indians/Asians is higher than in Europeans, Negroes, Whites and Mexicans (Parkin and Adams, 1975; Grunbaum et al., 1980; Divall, 1984) but lower than in Japanese (Omoto et al., 1975; Yoshida et al., 1979; Kido and Kimatsu, 1983; Tsutsumi et al., 1986). However the incidence of ESD^*2 allele among Indians from South East England is close to the findings of the present study (Parkin and Adams, 1975; Divall, 1984).

The laboratory prepared blood stains when exposed to ambient conditions showed typable enzyme activity upto 6-7 days in summer, 4-5 days in rainy season, 2-3 weeks in autumn and 4 weeks in winter months. When stored at -12°C, ESD isozyme patterns were detectable till 5 months. The stability pattern of this enzyme has also been reported by other workers; three weeks and two months old actual case materials (Parkin and Adams, 1975); 4 weeks and sometimes upto two months old stains (Grunbaum et al., 1978); 4 weeks (Jay and Philip, 1979); one month (Hayword and Bosworth, 1975); 8-10 weeks (Divall, 1985) and 5 weeks (Yuasa et al., 1985). As is evident from above the enzyme is comparatively less stable when exposed to Indian conditions of storage. Maximum stability could be achieved in winter months only when temperature touched as low as 6°C ± 2°C. Rapid loss of activity could be due to the fact that hot and humid conditions prevailing in our country

in the months of summer and rainy seasons could possibly be the reason for shortening the typable limits.

Liquid semen samples displayed all the three polymorphic forms of the enzyme (Table 2). In all instances, semen samples gave positive results. A few whole semen samples were centrifuged immediately after collection and two fractions, namely seminal plasma and spermatozoa were separated. Although both the fractions of semen exhibited genetic polymorphism but more intense band patterns were seen in sperms as compared to those in seminal plasma. The results from semen showing the presence of ESD activity are in accordance with the observations of Blake and Sensabaugh (1978). Further, liquid semen on storage at -12°C demonstrated typable activity upto 3-4 weeks. However when stains of semen were kept at room temperature in winter months, ESD patterns were identifiable upto 16-17 hours only. Jay and Philip (1979) observed the similar behaviour of this enzyme and found that activity declined considerably within 24 hours making ESD patterns too weak to interpret. It is apparent from the above that esterase D enzyme activity in semen is not as stable as in blood. Blake and Sensabaugh (1978) also reported that semen had moderate ESD activity and could be typed only under favourable circumstances.

Table 2: ESD activity in a matched sample of blood and semen

Samples Tested	Esterase D phenotypes		
	1	2-1	2
49 (fresh lysates)	36 (73.47)	11 (22.45)	02 (4.08)
49 (liquid semen)	36 (73.47)	11 (22.45)	02 (4.08)

Figures in parentheses indicate the percentages

Vaginal secretion samples collected from different donors exhibited esterase D polymorphism (Table 3). The band intensity was intense, suggesting high level of enzyme associated with vaginal fluid. Positive results could be obtained in about 41 percent of the cases. The findings of the present study are in general agreement with that of Sato et al. (1983) and Garlo (1985). When vaginal specimens were kept at room tempera-

ture typable activity could be achieved upto three days during winter months and 4 weeks when stored in a deep freezer at -12°C . Further, vaginal swabs collected from six student donors, who never had any sexual intercourse, showed weak enzymatic activity and no reliable typing was possible. Whether sexual stimulation or the presence of other body fluid (semen) has any role in enhancing the levels of enzymatic activity in vaginal secretions needs to be investigated in a larger sample.

Table 3: ESD activity in a matched sample of blood and vaginal secretions

Samples Tested	Esterase D phenotypes		
	1	2-1	2
64 (fresh lysates)	42 (65.63)	18 (28.13)	04 (6.25)
64 (vaginal secretions) +ve *	16 (25.00)	08 (12.50)	02 (3.13)

Figures in parentheses indicate the percentages.

*38 (59.37 %) samples of vaginal secretions gave negative results

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