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Stability Studies on ACP1 Isozymes in Human Blood and Menstrual Blood Stains: Polymorphism in a Heterogeneous Indian Population Sample

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ABSTRACT Enzyme typing of fresh lysates and their stains was performed on a heterogeneous Indian population sample. The polymorphic forms namely *A/ *A, *A/ *B, *B/ *B and *C/ *B and their gene frequencies were determined. Studies on paired samples of menstrual bloodstains along with donors' blood showed identical patterns in the zymogram. Laboratory prepared bloodstains on contamination with semen yielded inconsistent results.

INTRODUCTION

The genetic polymorphism of red cell phosphates (ACP1, EC 3.1.3.2) in man was discovered by Hopkinson et al. (1963) and was the first polymorphism which came to light during a survey to estimate the extent of inherited variations of human isoenzymes in normal healthy individuals (Hopkinson, 1985). Physiologically, two different functions have been proposed for ACP1: Flavin mononucloetide (FMN) phosphatase phosphotyrosine phosphatase (PTPase). Given that genetic variants of ACP1 activity are common, the enzyme could have a role in regulating a wide variety of cellular functions and, in turn, disease susceptibility (Dissing, 1993; Bottini et al., 1995).

Six common ACP1 phenotypes, designated *A/ *A, *A/ *B, *B/ *B, *A/ *C/, *B/ *C and *C/ *C can be recognized in most of the human populations by starch gel electrophoresis and are controlled by three autosomal alleles: *ACP1*A*, *ACP1*B*, and *ACP1*C*. The ACP1 locus has been assigned to the short arm of chromosome 2.

ACP1 is present in all tissues and is closely related to the cytosolic low molecular weight (cLMW) and phosphatases isolated from other vertebrate species (Dissing and Johnson, 1990, 1992; Wo et al., 1992a).

Each human homozygous type of ACP1 *A/ *A, *B/ *B, and *C/ *C, shows two main isozymes, designated 'f' and 's' according to their relative fast or slow anodal electrophoretic mobility. The f/s ratios of their activity are markedly different among genotypes and account for the phenotypic differences in activity modulation.

MATERIALS AND METHODS

Blood samples were collected from volunteer donors at NICFS representing almost all parts of India, and blood bank of Indian Red Cross Society, New Delhi. Stains were made on cotton cloth pieces. Dry bloodstains were cut in two parts, one stored at room temperature and another at -12°C in separate envelopes. Menstrual bloodstains were collected in cotton cloth/ swabs from donors along with donors' blood and stored as above. Semen drops were put on bloodstains to observe the effect of the contamination. For the electrophoretic analysis, the method of Wraxall and Emes (1976) was used with slight modifications (Sharma et al., 1985).

RESULTS AND DISCUSSIONS

Table 1 shows the number of ACP1 variants observed and allelic frequencies. All the three alleles are expressed in Indian population. The gene frequencies estimated for *ACP1*A*. *ACP1*B* and *ACP1*C* were 0.251, 0.746 and 0.003, respectively. The observed number of phenotypes was in good agreement with the number expected in sample population under

Table 1: Erythrocyte acid phosphatase enzyme distribution in a heterogeneous Indian population sample

Phenotypes	*Observed Number*	*Expected Number*	*Gene Frequencies*
ACP A	15	12.58	*ACP * A* = 0.251
ACP B	110	106.28	*ACP * B* = 0.746
ACP BA	67	73.14	*ACP * C* = 0.003
ACP CB	01	01.00	
Total	193	193	

χ^2 = 1.102, 0.30> p > 0.20; ns

Hardy- Weinberg equilibrium. Although *ACP1*A* and *ACP1*B* alleles are fairly well distributed in India, the frequency of *ACP1*C* allele is very low. The scenario is not much different in other Indian ethnic groups (see Bhasin et al., 1992) and as revealed by various others studies on viz. Black Africans and New Guineans (Hopkinson, 1968), Australian aborigines (Kirk et al., 1969), Japanese (Omoto and Harada, 1969; Kido and Komatsu, 1983), Turks and Germans (Weissman et al., 1980) and Whites and Blacks of United States (Budowle et al., 1985).

Blood stains stored under varied conditions were analysed periodically and the results showed that the stains kept at room temperature continued to give typable activity up to 4-6 weeks in winter, 8-12 days in summer, 5-7 days in rainy and 3-4 weeks in autumn months. The stains stored at -12°C could be successfully typed even after six months. Though enzyme typing from stains was non-problematic, yet storage bands were observed in the zymograms of aged stains. Hence prolonged treatment with Cleland's reagent of all the bloodstains was necessitated. The enzymatic activity gradually diminished with age. The loss of activity was faster in A isozyme than B and C. Pawlowski et al. (1990) also observed similar behaviour of this enzyme and considered it to account for the lower activity of this isozyme in red blood cells. Therefore care has to be exercised while interpreting results of aged bloodstains.

Menstrual bloodstains were analysed alongwith donors' blood (Table 2). Their zymogram pattern, movement and intensity were similar to those of the corresponding finger-prick blood samples of the respective donors. All the samples tested for the genetic marker could be typed without any ambiguity. Stability studies of stains were also carried out under varied storage conditions and the enzyme was found to be quite stable. The results did not show much variance from bloodstains in terms of typable limits.

Table 2: ACP Isozymes in Peripheral Blood and Menstrual Stains

Total Samples	*Erythrocyte acid phosphatase phenotypes*		
	A	*BA*	*B*
50 (fresh lysates)	3 (6.00)	16 (32.00)	31 (62.00)
50 (menstrual blood stains)	3 (6.00)	16 (32.00)	31 (62.00)*

* Figures in parentheses indicate the percentages

Paired samples (ten in number) of bloodstains and semen-contaminated blood stains were analysed to see the possible effect of one body fluid on the typing of the other. However, it was not possible to obtain reliable results in contaminated stains owing to interference. Only intense trailing of enzyme activity was observed. Typing for ACP isozymes in such cases therefore is not recommended.

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