Placental enzyme polymorphism among Maharashtrians: Alkaline phosphatase and lactate dehydrogenase

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Summary. The distribution of placental alkaline phosphatase and lactate dehydrogenase types in 635 placentas from various endogamous groups of Maharashtra have been studied by starch gel electrophoresis. In the case of alkaline phosphatase, 6 common phenotypes and 6 rare phenotypes $\{F_2I_1, S_1S_2, S_2S_3, I_1S_2, F_1I_2\}$ are encountered. The highest frequency of P^{I_2} allele $\{0.7394\}$ and lowest frequency of P^{I_2} allele $\{0.7394\}$ and lowest frequency of P^{I_2} allele $\{0.7394\}$ and seases of Cal-1 and 5 cases of Cal-2 types of LDH variants have been observed in the total samples, and Muslims possess the highest frequency of Cal-1 types $\{3.64\%$. Population groups are compared with respect to P^I alleles.

1. Introduction

Very little is known about the distribution in India of the considerable polymorphism which has been detected in placental alkaline phosphatase (E.C.3.1.3.1.) (Robson and Harris, 1967; Blake et al., 1969 a; Das et al., 1970, 1974) and placental lactate dehydrogenase (LDH E.C.1.1.1.) (Das et al., 1972). The aim of this contribution is to help remedy this deficiency.

2. Materials and methods

The present study was undertaken in Maharashtra during 1973-74 among various endogamous groups of Marsthi-speaking people. A total of 630 placenta samples were collected from the following groups: Brahmin (51), Marsthas (208), some mixed "middle castes" (46), Nava Budha (Mahar) (142), some mixed "low castes" (41), Muslims (54) and other non-Marathi-speaking Indians (88). According to the traditional caste hierarchy followed in Maharashtra, the above groups may be ranked as follows: the Brahmins represent the higher caste, the Marathas belong to middle castes and the Nava Budha is a scheduled caste. Besides these major caste groups, many other castes in small numbers come into the sampling and, for convenience of analysis, they are broadly classified into two caste groups—"middle castes" (Dhangars, Mali, Sali, Shimpi, Nahvi, Koshti, Parit, Gosavi, Teli, Lohar, Kali, Sonar, Sutar, Kumbhar, Bhandari etc.) and "low castes" (Chambhar, Wadars,

		;		O	common p	henotypes	_				Rare pbe	notypes			
Population		No. tested	SıSı	F,F,	1,1,	S,F,	S,I,	F,I,	SiSz	S,S,	F,S,	I,S,	F,I,	Fils	D.F. 12
Brahmin	≥%	51	24 47 · 06	3.92	90	21 41 - 18	3.88	800	8		8	8	8.	- <u>;</u> -	4-335
Maratha	%≼	208	105 50:48	13 6:23	- 0 - 48	61 29-33	9.13	6 2·89	6 5 9 9	9	8		8 0	800	4.908
Middle caste	>%	\$	28 60·87	4 % 2	3 6·52	15:22	2 4·35	800	4.35			800	900	800	35.008
Nava Budha (Mahar)	≥%	142	78 54·93	6.3 4	800	47 33·10	4.93	0 0	8				8	900	2-956
Low caste	۶%	4	17 41 - 46	9 · 76	8	39·05	3.	800	8				- 5 4 4	8	19-961
Muslim	≥ %	ž	28 51-86	- 8 2	3.7 5.7	14 25:93	8.58 8.78	- 82	4.5 5.70				8	800	2.768
Others	≥%	8	46 52·27	2.27	2.27	30 34·09	4.55	4.55	00.00				8	00.0	10 · 789

Table 1. Placental alkaline phosphatase phenotype distributions in various populations of Maharashtra.

Bhoi, Gurav, Bhangi, Mang, Rameshi, Harijan etc.). The position of the Muslims is self-explanatory. All these samples were collected from B.J. Medical College and Sasson Hospital, and Bhidya Maternity Home in Poona.

The preparation of placental tissue extract and the starch gel electrophoresis technique for both alkaline phosphatase and LDH were performed as described by Blake et al. (1969 a, b).

3. Results and discussion

Phenotypes (alkaline phosphatase)

The distribution of the various phenotypes in the different population groups are listed in Table 1. Expected phenotype numbers in each group are calculated assuming Hardy-Weinberg equilibrium and the χ^2 values are given in the same table. From these values it is clear that in all the groups there is close agreement between the expected and the observed distribution, except in the mixed middle castes; where the value is significantly high ($\chi^2 = 35.008$); this may be due to the heterogeneous composition of the group.

In all the groups, five out of the six common phenotypes are observed. The I_1I_1 type is not found in the Brahmin, the Nava Budha and the low castes, but is present in all the other groups. Five rare phenotypes S_1S_2 , S_2S_3 , F_2I_1 , F_2S_3 and I_1S_2 have been found sporadically. In addition, a new phenotype, which has been identified as F_1I_2 corresponding to another rare allele $P_1^{I_2}$, has also been encountered in the Brahmin group (one case in a sample of 51). The I_2 electrophoretic band in F_1I_2 pattern is slower than the I_1 band but definitely faster than the S_1 band.

In most of the groups approximately 50% are homozygous S₁S₁ type; the exceptions are the mixed "middle castes" (60·87%) and mixed "low castes" (41·46%). Next in order of preponderance in all the groups is the heterozygote S₁F₁.

Gene frequencies

Gene frequencies are calculated by the gene counting method and Table 2 presents the distribution in the various groups. The three common alleles $P_i^{\mu_1}$, $P_i^{\mu_1}$, and $P_i^{\mu_2}$ are present in all the groups and the most widely distributed allele $P_i^{\mu_1}$ ranges between 0.7394 (Nava Budha) and 0.6463 (mixed "low castes"). Rare alleles $P_i^{\mu_2}$, $P_i^{\mu_3}$, $P_i^{\mu_1}$ and $P_i^{\mu_2}$ have also been detected in very low frequencies. The homogeneity between the population groups has been tested by considering the actual gene count

	Co	ommon alk	eles					
Population	Pl*1	Pl'1	Pi¹₁	Pl*2	Pl's	Pl¹s	Pila	Total
Brahmins	0.7059	0.2549	0.0294	0.0000	0.0000	0.0000	0.0098	1.0000
Marathas	0.7010	0.2170	0.0660	0.0160	0.0000	0.0000	0.0000	1.0000
Middle castes	0.7134	0-1431	0.0771	0.0664	0.0000	0.0000	0-0000	1-0001
Nava Budhas	0.7394	0.2324	0.0246	0.0035	0.0000	0.0000	0.0000	0.9999
Low castes	0.6463	0.2927	0.0488	0.0000	0-0000	0.0122	0.0000	1.0000
Muslims	0.6890	0.1500	0.0900	0-0620	0.0090	0.0000	0.0000	1.0000
Others	0.7159	0.2159	0.0682	0.0000	0.0000	0.0000	0-0000	1.0000

Table 2. Placental alkaline phosphatase gene frequencies in various populations of Maharashtra.

of the alleles instead of the absolute phenotype or the genotype frequencies. From Table 3 it appears that the frequencies of Pl^{i_1} gene count is very small in all the population groups and the rare alleles are also too small for any valid comparison. In order to determine the homogeneity between the population groups, parallel sample chi-square tests have been performed based on the gene counts of Pl^{i_1} allele, Pl^{i_1} allele and all the rare alleles. It is seen that even when the gene count of the alleles Pl^{i_1} and all the rare alleles were added together, the total frequencies of this class were not large for all the population groups and five significant chi-square values (with 2 d.f.) were obtained. We have, therefore, attempted two other series of parallel sample tests as follows. The first series based on the gene count of the alleles Pl^{i_1} and the gene count of all the other alleles (i.e. Pl^{i_1} , and rare alleles) added together, and the second was based on the gene count of Pl^{i_1} and the gene count of all other alleles (i.e. Pl^{i_2}), and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}) and the gene count of all other alleles (i.e. Pl^{i_3}).

Population	Pl*1 count	Pl'1 count	Pl'1 count	All rare alleles count	Total
Brahmin					
Obs.	72	26	3	1	102
Exp.	72 · 54	22-68	5-64	1.13	101-99
Maratha					
Obs.	292	93	28	3	416
Exp.	295.88	92.49	23.03	4.60	416
Middle caste					
Obs.	67	15	8	2	92
Exp.	65-44	20-45	5.09	1.01	91.99
Nava Budha					
Obs.	210	66	7	1	284
Exp.	201 - 99	63 - 14	15.72	3.14	283-99
Low caste					
Obs.	53	24	4	1	82
Exp.	58-32	18 · 23	4-54	0.91	82.00
Muslim					
Obs.	77	17	10	4	108
Exp.	76-82	24.01	5.88	1.19	108

Table 3. Distribution of PI alleles by gene count method in various populations of Maharashtra.

From the chi-square values obtained by the above method, it may be concluded that (1) there is not much heterogeneity among the groups for different Pl gene counts; (2) significant differences are noticed only when the Muslims are compared with Nava Budhas, the "low caste" and the Brahmin groups for Pl^{n_1} , Pl^{n_1} and the "remainder" alleles ($\chi_2^2 = 16.50$, 6.45 and 7.44 respectively); (3) similarly, the Nava Budhas also differ from the Marathas and the "mixed" middle castes ($\chi_2^2 = 6.89$ and 10.97 respectively); (4) significant variation is also revealed when the low caste group is compared with the "mixed" middle caste and the Muslims with respect of Pl^{n_1} and the "remainder" alleles ($\chi_1^2 = 4.19$ and 5.04 respectively); (5) but complete homogeneity is found amongst all the groups when compared for Pl^{n_1} and the "remainder" alleles.

Lactate dehydrogenase

LDH isozymes were typed against known control variants and the distributions of various LDH variants, and their gene frequencies are given in Table 5. The incidence of Cal-1 type, which is a faster A-sub-unit variant, has been noticed in the Nava Budha (about 2%) and the Muslims (about $2\cdot6\%$) and other non-Maharashtrians (about 1%). But the Cal-2 variant (much faster A-sub-unit variant) is present in the Brahmins, the Marathas, the "middle caste" and the Nava Budhas in relatively low frequencies (1-2%).

Population	x ² test for Pl*1, Pl*1 and all other alleles count (2 d.f.)	χ² test for Pl*1, and all other alleles count (1 d.f.)	χ² test for Pl¹1, and all other alleles count (1 d.f.)
BRA×MAR	1 · 8631	0.0061	0.4548
BRA×MID	5.2008	0.1192	2 · 4488
BRA×NAV	0.5697	0.4292	0 · 2094
BRA×LOW	0.9160	0.7397	0.3278
BRA×MUS	7 - 4417†	0.0127	3.0621
MAR×MID	2.4619	0-2521	1 - 6480
MAR×NAV	6·8972†	1 · 1708	0.0750
MAR×LOW	1.8724	0.9942	1 · 8209
MAR×MUS	4.8344	0.0501	2 · 2621
MID×NAV	10-97581	0.0447	1.9773
MID×LOW	4.8181	1 - 3594	4·1901†
MID×MUS	0.2074	0.0576	0.0117
NAV × LOW	3 · 6359	2 · 7269	1 - 2471
NAV × MUS	16.50491	0.2796	2.6361
LOW × MUS	6-4519t	0.9574	5·0403†

[†] Significant at 5% level, but insignificant at 1% level.

Table 4. Inter-group comparison with respect to Pl allele gene count amongst various populations of Maharashtra.

				Phenotype		G	ene frequenc	ies
Population		tested	Normal	Cal-I	Cal-2	LDH*	TDH _{C1-1}	LDHC
Brahmin	N %	49	48 97·96	0	1 2·04	0.9898	0.0000	0.0102
Maratha	<i>N</i> %	209	209 99·52	0	1 0·48	0.9976	0.0000	0.0024
Middle castes	N %	47	46 97 - 87	0	1 2·13	0.9893	0.0000	0.0107
Nava Budhas	N %	144	139 96·53	3 2·08	2 1 · 39	0.9826	0.0104	0.0070
Low castes	N %	41	41	0	0	1.0000	0.0000	0.0000
Muslims	N %	55	53 96·36	2 3·64	0	0.9818	0.0182	0.0000
Others	N %	90	89 98 · 89	1 1-11	0	0.9945	0.0056	0.0000

Table 5. Distribution of lactate dehydrogenase types and gene frequencies in various populations of Maharashtra.

^{\$} Significant at 0.1% level.

BRA = Brahmin, MAR = Maratha, NAV = Nava Budha, MID = Mixed middle caste, LOW = Mixed low castes. MUS = Muslim.

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References

- Blake, N. M., Kirk, R. L., and Mehra, B. (1969 a). Placental alkaline phosphatase types in Malaysia. Human Heredity, 19, 20-24.
- Blake, N. M., Kirk, R. L., Pryke, E., and Sinnet, P. (1969 b). Lactate dehydrogenase electrophoretic variant in a New Guinea Highland population. Science, 163, 701.
- Das, S. R., Mukherjee, B. N., Das, S. K., Blake, N. M., and Kirk, R. L. (1970). Placental alkaline phosphatase types in Calcutta. *Japanese Journal of Human Genetics*, 15, 155-158.
- Das, S. R., Mukherjee, B. N., and Das, S. K. (1972). Caste and age variation of the incidence of LDH variants in the Bengali Hindus. Humangenetik, 14, 151-154.
- Das, S. R., Mukherjee, B. N., and Das, S. K. (1974). Caste variation in the distribution of placental alkaline phosphatase genes among the Hindus of West Bengal. Annals of Human Biology, 1, 65-71.
- Robson, E. B., and Harris, H. (1967). Further studies on the genetics of placental alkaline phosphatase. Annals of Human Genetics, 30, 219-232.

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Zusammenfassung. Die Verteilung der Typen der placentalen Alkali-Phosphatase und LactaDehydrogenase wurde bei 635 Plazenten von verschiedenen endogamen Gruppen von Maharashtras
durch Stärkegel-Elektrophorese untersucht. Bei der Alkalin-Phosphatase wurden 6 übliche Phäno
typen und 6 seltene Phänotypen (F₁I, S,S), S,S, I,S, F,S, F,F), pglunden. Die höchste Frequenz
des Allels PP1 (0,7394) und die niedrigste Frequenz von PP1 (0,0246) wurden bei dem Nava-Budha
gefunden. Sechs Fälle von Cal-1 und fünf Fälle von Cal-2 wurden bei den LDH-Varianten in der
gesamten Stichprobe gefunden, und die Moslems besitzen die höchste Häufigkeit des Typs Cal-1
(3,64%). Ein Vergleich zwischen Bevölkerungsgruppen wurde für das Allel Pl durchgeführt.

Résumé. La distribution des types de phosphatase alcaline et de lactate déshydrogénase placentaires a été étudiée dans 635 placentas provenant de divers groupes endogames de Maharashira par électrophorèse sur gel d'amidon. Dans le cas de la phosphatase alcaline, 6 phénotypes communs et 6 phénotypes rares (F,11, S,5, S,5), 1,5), F,S, F,I,) sont rencontrés. La fréquence la plus élevée de l'allèle Pl¹ (0,0246) ont été trouvées chez les Nava-Budha. Six cas de types Cal-1 et 5 cas de Cal-2 des variantes de LDH ont été observés dans les échantillons totaux, et les Musulmans ont la fréquence la plus élevée de types Cal-1 (3,64%). La comparaison a été faite entre les groupes de populations en ce qui concerne les allèles Pl.