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Screening for Haemoglobin Variants in People of Punjab, North-West India

S.M.S. Chahal, Harjot Pal Kaur, Rupinder Kaur Virk and Harjeet Singh

Department of Human Biology, Punjabi University, Patiala 147 002, Punjab, India

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ABSTRACT Haemoglobin variation was studied in different endogamous caste populations and Muslim of Punjab in conjunction with erythrocyte enzyme glyoxalase (GLO) typing of 3,401 haemolysates. In all 40 haemoglobin variants were encountered, giving an incidence of little over 1% for people of this North-West border Indian state. Screening for haemoglobin variants by this novel method is recommended in all routine genetic marker surveys from India that investigate GLO polymorphism, among others.

INTRODUCTION

Haemoglobin, one of the best known blood proteins, gives rise to the most thoroughly studied genetic polymorphism in man - the polymorphism that includes the gene for sickle cell anaemia (Cavalli-Sforza and Bodmer, 1971). The study of this red cell protein has made several significant contributions toward the development of molecular biology.

Pauling et al. (1949) demonstrated that presence of an abnormal haemoglobin S (Hb S) having an altered electrophoretic mobility than normal haemoglobin A (Hb A) was responsible for sickle cell anaemia. Hb S differed from its normal counterpart (Hb A) by a single amino acid substitution in the beta polypeptide chain (Ingram, 1957). Like Hb S, most abnormal haemoglobins (including Hb C, Hb D, Hb E) are products of point mutation and their detection is dependent on a difference in their altered electrophoretic mobility. Worldwide more than 470 genetically controlled haemoglobin variants, mostly of the beta chain, are known (Honig and Adams III, 1986). Besides their clinical importance, they serve as genetic markers for human population variation studies, especially in the tropics.

Divall and Greenhalgh (1983) demonstrated that blood samples can be screened effectively for the common haemoglobin variants (Hb AS, AC, AD and AE) in conjunction with glyoxalase (GLO) typing and only variants so detected be further identified. This provided an excellent opportunity for any enzyme electrophoresis laboratory, such as the present, which routinely types large number of blood samples in the GLO system to additionally screen them for haemoglobin variation without any additional efforts and great savings in time and expenses. In fact, using their method, even in forensics haemoglobin variants in blood as well as bloodstains can be detected reliably by simply observing for haemoglobin pattern on glyoxalase electrophoresis plates. Such information could be of considerable evidential value, especially when the case material is limited and requires judicious usage.

With its very large and ethnically diverse population inhabiting different ecological/ geographical zones, India offers a rewarding opportunity for screening haemoglobin variants. Since the enzyme electrophoresis laboratory at Patiala has been actively engaged in various biochemical genetic investigations among people of North and North-West India over the last over one decade now, it is in a unique position to generate large haemoglobin variation data on populations of different states in these parts of the country. This report provides such data among as many as 24 different endogamous caste/ religious groups of Punjab, in the first instance.

SUBJECTS, MATERIAL AND METHODS

The present study is based on observations made on glyoxalase (GLO) electrophoresis plates of a total of 3,401 fresh haemolysates

from Punjab. The subjects were apparently healthy not closely related school going boys and girls inhabiting Ferozepur, Faridkot, Bathinda, Sangrur, Rupnagar and Patiala districts of the state. The enzyme typing was carried out in mixed agarose/starch gels on glass plates following the technique standardized in this laboratory and described elsewhere (Chahal et al., 1986; Bhasin and Chahal, 1996).

Prior to staining for GLO, all plates were carefully visually inspected for the haemoglobin pattern. The usual pattern, a single major slow moving band towards cathodal side, almost on origin, was recorded as 'normal'. Any double banded pattern, comprising of a faster moving major band towards cathode and a usual slow moving major (normal) band, was recorded as a 'variant'. No fast moving single major band pattern (corresponding to homozygous variant) was found. All haemoglobin variants were repeat run for confirmation; all were clearly distinguishable from the control normal (Hb AA) pattern and there was no ambiguity in any case. However, no effort was made to further identify the variant pattern encountered in this survey.

RESULTS AND DISCUSSION

Details of areas and populations tested from Punjab for haemoglobin types in this study are set out in table 1. This table shows that as many as 17 regional population groups of the Jat Sikh, Ramdasia and Majhbi from all the six studied districts, some other castes (*viz.*, the Kamboh, Brahmin, Khatri, Bania and Ramgarhia Sikh) from Patiala district and two Muslim sects from Sangrur district were screened from the plateau area of Malwa- a geographical region of the state lying south of river Satluj.

In 34 hundred-odd haemolysates from Punjab, primarily electrophoresed for glyoxalase typing and screened for haemoglobin variation, a total of 40 cases of double banded pattern were detected, giving a frequency of 1.2% for the haemoglobin variants in the state. It is interesting to note that these variants, encountered in almost two-thirds of the 24 groups studied here, were present both in the caste material as well as the Muslim population of Punjab. When present, the incidence of haemoglobin variants in the state ranged from 0.5% in the Shia Muslims of Sangrur district to 4.8% in a small sample of Ramgarhia Sikh investigated from Patiala district (Table 1).

Among people of Punjab, sickle cell examination studies on erythrocytes revealed absence of sicklers (Aksoy et al., 1955; Saha and Banerjee, 1965; Chaudhuri et al., 1967; Pandey et al., 1970). Apparently, this implied absence of Hb S in the state. On the other hand, electrophoretic analysis of haemolysates by some others (Lehmann, 1956-57; Bird and Lehmann, 1956; Bird et al., 1956; Saha and Banerjee, 1965, 1971; Papiha, 1973), did prove beyond doubt that abnormal haemoglobins, including Hb S, were indeed present in populations of Punjab (Table 2). Out of 1454 subjects in these studies,

Table 1: Haemoglobin types among various endogamous population groups of Punjab

Area/Population	*N*	*Haemoglobin type*	
		Normal	*Variant*
Ferozepur District			
Jat Sikh	140	140	-
Ramdasia	86	86	-
Majhbi	114	111	3 (2.7)
*Faridkot District**			
Jat Sikh	141	137	4 (2.8)
Ramdasia	82	80	2 (2.4)
Majhbi	123	121	2 (1.6)
Bathinda District			
Jat Sikh	157	156	1 (0.6)
Ramdasia	103	103	-
Majhbi	96	96	-
Sangrur District			
Jat Sikh	164	160	4 (2.4)
Ramdasia	119	119	-
Majhbi	92	89	3 (3.3)
Sunni Muslims	803	793	10 (1.2)
Shia Muslims	189	188	1 (0.5)
Rupnagar District			
Jat Sikh	168	168	-
Ramdasia	127	125	2 (1.6)
Majhbi	76	76	-
Patiala District			
Jat Sikh	54	53	1 (1.9)
Kamboh	170	170	-
Brahmin	82	82	-
Khatri	143	139	4 (2.8)
Bania	110	109	1 (0.9)
Ramgarhia Sikh	21	20	1 (4.8)
Ramdasia Sikh	41	40	1 (2.4)
Punjab	3401	3361	40 (1.2)

Figures in parentheses are percentages
* Sampling from area now falling under Muktsar district

a total of 24 haemoglobin variants were found, giving an overall incidence of 1.7%. The incidence of haemoglobin variants in the present study, which has added information on further 40 variants in 3401 subjects, has been recorded 1.2%. These two estimates are similar and represent the true extent of haemoglobin variation in Punjab. By comparison to the average value quired.

Finally, the importance of the method of Divall and Greenhalgh (1983) regarding screening of haemoglobin variation needs to be emphasized, since it is of special interest for laboratories in tropical countries in which most of haemoglobin variants reported in the world have been found. Thus, now there is an oppor-

Table 2: Haemoglobin variants reported in Punjab

Area/Population	*N*	*Haemoglobin Variants*						*Reference*
		AS	*AD*	*AE*	*AF*	*DD*	*Total*	
Sikhs	62		1	1			2 (3.2)	Lehmann, 1956-57
Sikhs	109		1				1 (0.9)	Bird & Lehmann, 1956
Sikhs	279		4			1	5 (1.8)	Bird et al., 1956
Punjabi Hindus	13		1				1 (7.7)	Bird et al., 1956
Ludhiana								
Punjabi Hindus	225		1				1 (0.4)	Saha & Banerjee, 1965
Sikhs	125		3				3 (2.4)	Saha & Banerjee, 1965
Sikhs	378		5			1	6 (1.6)	Saha & Banerjee, 1971
Punjabi Hindus	123		1		1		2 (1.6)	Saha & Banerjee, 1971
Chandigarh								
Punjabi	140	3					3 (2.1)	Papiha, 1973
Total	1454	3	17	1	1	2	24 (1.7)	

Figures in parentheses are percentages

of 0.5% for Indian populations (Bhasin et al., 1994), the incidences of haemoglobin variants recorded in people of Punjab were rather high (Tables 1, 2).

Haemoglobin variants encountered in earlier studies from Punjab were mostly of Hb AD (or Hb DD) type and others were Hb AS, save one example each of Hb AE and Hb AF . However, since band patterns of Hb AD cannot be distinguished from that of Hb AS using the glyoxalase electrophoresis system, identity of 40 variants found in this screening study could not be revealed. Nonetheless, the present study has amply demonstrated that like in Sikh and Hindu religious and Punjabi linguistic groups (Table 2) abnormal haemoglobins are universally distributed even in various endogamous caste populations as well as the Muslim religious groups inhabiting the Malwa region in southern Punjab. However, to obtain a full picture of variation of this important blood protein in people of Punjab, such data from the two other geographical regions of the state lying north of the Satluj *viz*., the Majha and Doaba, are retunity for studying haemoglobin variation simultaneously with glyoxalase typing which must not be missed in any future study from India, for "India is a great reservoir for abnormal haemoglobins" (Saha and Banerjee, 1971). Although the method has some limitation, its usefulness lies in providing additional genetic information practically without any additional efforts or time.

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