A Correlative Study of HLA, Sickle Cell Gene and G6PD Deficiency with Splenomegaly and Malaria Incidence Among Bhils and Pawra Tribes from Dhadgon, Dhule, Maharastra

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INTRODUCTION

The tribal groups in India form a significant proportion (8.01%) and they are believed to be the earliest settlers or inhabitants of the region. There are 427 tribal communities in India (Singh, 1993). The dominant ethnic group among the Indian tribal group is proto-australoid although groups living in the sub-Himalayan belt have more of Asian characteristics. The three major tribes having a population of more than a million are Gonds of Madhya Pradesh, Maharastra and Andhra Pradesh, the Bhils of Rajasthan, Gujarat, Maharastra and Madhya Pradesh and the Santhals of Bihar, Orissa and West Bengal (Vidhyarthi, 1983). The Bhils are one of the largest tribes concentrated mainly in Western Madhya Pradesh, Rajasthan, Eastern Gujarat and Northern Maharastra. Racially they were classified as Gondids, Malids or Proto-Australoid, but their social history is still a mystery (Bhatia and Rao, 1986). The Pawras resemble a primitive tribe koli of Western Satpura Hills and are concentrated mainly in Khandesh. Even today these tribal groups hardly perform their marriages outside their tribe, which makes them unique from the cast groups of plains.

There are several reports in literature on population genetics that show prevalence of G6PD deficiency, Sickle cell trait frequency, in different caste groups (Baxi et al., 1963, 1964; Mehta et al., 1971; Bhasin et al., 1992; Joshi et al., 2001, Bhasin and Walter, 2001) and tribal groups (Rao and Bhatia, 1988; Gorackshekar et al., 1987; Bhasin et al., 1992; Kaur et al., 1997; Ramasamy et al., 1994) from India. Further there are reports on the genetic diversity of HLA in different tribal groups from India (Shankarkumar et al., 1999). But still there are very little information on the correlation of Sickle cell trait, G6PD deficiency and HLA in a caste/ tribal group from India where Malaria is endemic. This initiated us to undertake the current study among the Malaria endemic population of Bhils and Pawras from Dhadgon, Dhule district, Maharastra, Western India.

MATERIALS AND METHODS

Samples: A total of 146 tribal comprising Bhils (91) and Pawras (55) living in Dhadgon, Dhule district, Maharastra were studied for HLA, Sickle cell Trait and G6PD deficiency. Blood samples were collected in EDTA and analysis was done within 24 hours. They were clinically assessed by (KG) for the presence of splenomegaly and Malarial incidence by standard technique (John Mcleod, 1983).

HLA Typing: The two - stage NIH standard micro-lymphocytotoxicity test was carried out on all the samples as described elsewhere (Shankarkumar et al., 1999).

G6PD Assay: Screening for G6PD deficiency was done by the dichlorophenol-indophenol (DPIP) dye decolorization method (Bernstein 1962). The RBC indices and hematological parameters were estimated using an automated cell counter (ERMA - PC, 608, Japan). Hemoglobin analysis was estimated using the Variant hemoglobin testing system (Bio-Rad, Inc Ohio, USA) using recommended protocols. Solubility test and cellulose acetate electrophoresis using Tris - EDTA borate buffer at pH 8.9 (Dacie and Lewis, 1975) was also done.

RESULTS

HLA Antigen Associations, Sickle Cell Trait and G6PD Deficiency Comparisons: Analysis of the HLA antigens showed that in the splenomegaly and Malaria positive group HLA A1, B40 and B53 ($P < 0.001$) were increased when compared to the negative group where A2, B7 and B35 ($P < 0.01$) were decreased (Fig. 1). HLA A2, B8, B17 and B40 ($P < 0.001$) were significantly

increased in the G6PD deficient group when compared to the normal where A19, A11, and B53 (P <0.01) were decreased (Fig. 2). HLA A2, B40 and B53 (P<0.0001) were increased among the sickle cell heterozygotes when compared to the homozygotes where A19 and B8 (P< 0.05) were decreased (Fig. 3). Moreover the HLA distribution among the tribes were diverse from other reported tribal populations (Shankarkumar et al., 1999).

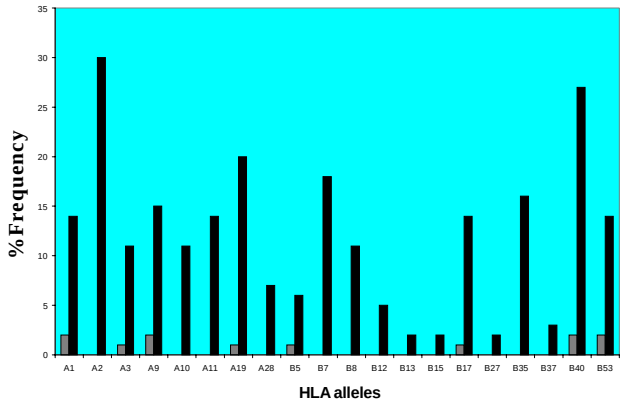
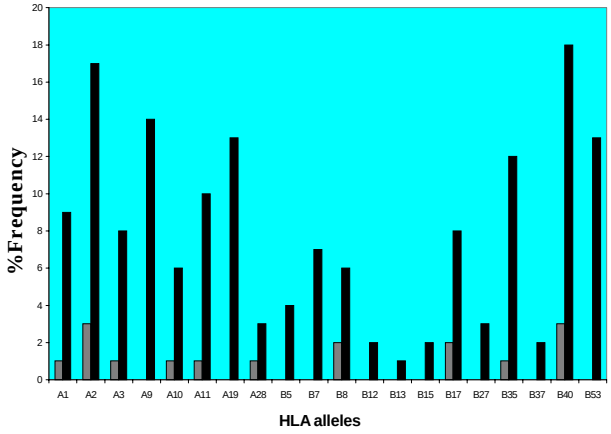


Fig. 1. Splenomegaly and Malaria

Fig. 2. Splenomegaly and Malaria



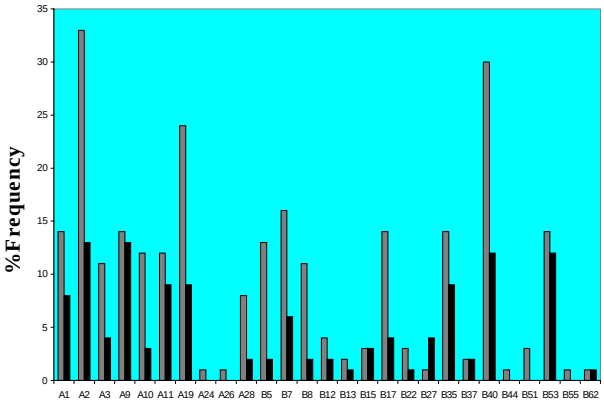


Fig. 3. HLA and Sickle cell status

DISCUSSION

The mortality caused by Malaria in high endemic parts of the world enabled the human survival to evolve a genetic trait, which can defend against malarial infection. These types of protective genetic measures have been observed with relation to polymorphism at the Sickle cell disease, G6PD and the HLA system (Hill et al., 1991, 1992). Such wide genetic variation in different populations has been a result of various factors like a) original gene pool b) migration and genetic drift c) gene selection under different environments and d) inbreeding pattern within the population (Bhatia, 1987). When a gene that has a potential for reducing fitness achieving high frequencies in a population it is necessary to assume the gene confers a survival advantage (Beutler, 1994). There has been significant differences in the HLA gene frequencies between malaria exposed and unexposed populations (Hill et al., 1991, 1992; Brown et al., 1989; Troye-Blomberg et al., 1991; Riley et al., 1991). Comprehensive analysis of HLA association with infectious diseases has allowed precise definition of susceptibility and protective alleles in large population of different ethnic groups. Infectious diseases may have exerted significant pressure on the development and maintenance of HLA polymorphism (Potts et al., 1990). Widespread and frequently fatal parasitic diseases such as Malaria have selectively maintained certain gene frequencies in endemic disease areas (Osaba et al., 1979). Further it has been speculated Sickle cell disease, G6PD deficiency and HLA polymorphism offers protection against malaria in a malaria endemic region.

Haldane proposed that the sickle cell gene provides an advantage in a malaria endemic region. Allison (1954) presented evidence for the balanced polymorphism of sickle cell gene and the sickle cell trait (heterozygotes) and G6PD has been suggested to provide resistance to falciparum malaria (Raper, 1955; Edington, 1965). The HLA polymorphism of Bhils and Pawras along with other Indian tribes (Selvakumar et al., 1987; Pitchappan et al., 1997; Paphia et al., 1983) revealed that all these tribes do not cluster together as a separate entity: suggesting that different tribes have different HLA profile, presumably because of their isolated origin. It is generally believed that the tribes were the early settlers in the Indian Plateau who were driven to the hills by ethnically distinct invading populations (Sanghvi et al., 1981). They have high inbreeding co-efficiency. In India many of the forest and hilly areas that are especially vulnerable are endemic for Malaria. With the current resurgence of malaria in India and its importance as a national health issue it is important to know the prevalence of genetic factors associated with malaria that are present in individuals living in areas endemic for the disease.

KEY WORDS Immunity; genetic markers; tribes; West India; environment

ABSTRACT Haldane proposed that the sickle cell gene provides a survival advantage in malarial endemic regions. A high malarial endemicity has also been attributed to a high prevalence of G6PD deficiency in the Middle East and South East Asia. Moreover, the HLA antigens have been postulated to confer a protective immunity among the malaria endemic population. To evaluate a correlation in between these investigations among 146 tribals comprising of Bhils (91) and Pawras (55) living in Dhadgoan, Dhule district of Maharastra where Malaria is endemic. It was interesting to note that HLA A1, A9, B5, B40 and B53 alleles where significantly increased among clinically splenomegaly positive patients with a history of Malaria when compared to normal controls (PF > 40% P < 0.0001). HLA A2, B8, B17 and B40 alleles were significantly increased among the G6PD deficient tribals when compared to non-deficient individuals (PF >30%, P< 0.001). Further, it was observed that B53 was significantly increased among the sickle cell hetrozygotes (PF > 45% P< 0.0001) of Bhils and Pawra tribes. A comparison of sickle cell trait and G6PD deficiency status with other Indian tribal populations reported revealed that in our study a 9% G6PD deficiency and 31 % sickle cell heterozygotes with a 17% Sickle cell disease among the tribal population studied. It will be of great interest if we further characterize the HLA, G6PD and Sickle cell gene by molecular methods to study the influence of various environmental factors like hilly terrain, natural calamities and epidemics in the prevalence of Malaria and its association with these parameters at a molecular level among these tribes.

REFERENCES

Allison, A.L. 1954. “Protection afforded by Sickle cell trait against subtertian Malaria infection.” *Br. Med. J.*, 1: 290-294.

Baxi, A.J., J.V. Undevia and H.M. Bhatia 1964. “Role of G6PD deficiency in acute hemolytic crisis and neonatal jaundice.” *Ind. J. Med. Sci.*, 18: 57.

Baxi, A.J., K. Balakrishnan, and J.V. Undevia 1963. “G6PD deficiency in Parsee community.” *Ind. J. Med. Sci.*, 17: 493-500.

Bernstein, R.E. 1962. “A rapid screening dye test for the detection of G6PD deficiency in red cells”. *Nature*, 194: 192-193.

Beutler E. 1984. “G6PD deficiency”. *Blood*. 84(11): 3613-3636.

Bhasin, M.K., and H. Walter, H. Dauker-Hopfe. 1992.

The Distribution of Genetical, Morphological and Behavioural Traits Among People of Indian Region. Delhi : Kamala-Raj Enterprises.

Bhasin, M. K. and H. Walter. 2001. *Genetics of Castes and Tribes of India.* Delhi: Kamla-Raj Enterprises.

Bhatia, H.M. 1987. "Genetic parameters: Biologic and Epidemiological Significance." *Ind. J. Med. Sci.,* 41: 203-206.

Bhatia, H.M. and V.R. Rao. 1986 *Genetic Atlas of Indian Tribes.* Institute of Immunohamatology, New Delhi: ICMR Publications.

Brown, A.E., D. Chandanayingyong, S.V. Fuggle and H.K. Webster. 1989. "Immune response to the circumsporozoite protein of *Plasmodium falciparum* in relation to HLA DR type." *Tissue Antigens,* 34: 200-204

Clinical Examination. 1983. *The Alimentary and Genito Urinary system.* Edingburg: Churchill Livingstone, 206.

Dacie, J.V. and S.M. Lewis 1975. *Practical Hematology* 5th Ed. London: Churchill Livingstone. 227.

Edingten, G.M. and E.J. Watson-Williams. 1965. "Sickling, Haemoglobin C, Glucose – 6- phosphate dehydrogenase deficiency and malaria in Western Nigeria". In: *Abnormal Haemoglobin in Africa*. J.H.P. Jonix (Ed.). Oxford: Blackwell Scientific Pub. 393.

Goracksha kar, A.C., M.S. Sathe, S.R. Shirsat and H.M. Bhatia. 1987. "Incidence of ABO, Rho (D), In[a] antigens, G6PD deficiency and abnormal haemoglobins". *J. Ind. Anthropol. Soc*., 22: 38-46.

Hill, A.V.S., C.E.M. Allospp, D. Kwiatkowski, N.M. Anstey, P. Twumasi, P.A. Rowe, S. Bennet, D. Brewster, A.J. McMichael and B.M. Greenwood. 1991. "Common west African HLA antigens are associated with protection from severe Malaria." *Nature,* 352: 595-600.

Hill, A.V.S., J. Elvin, A.C. Wills, M. Aidoo, C.E.M. Allospp, F.M. Gotch, X.M. Gao, M. Takiguchi, B.M. Greenwood and A.R. Townsend 1992. "Molecular analysis of the association of HLA B53 and resistance to severe Malaria." *Nature,* 360: 434-439.

Joshi, S.R., R.Z. Patel, H.R. Patel, S. Sukumar and R.B. Colah. 2001. "High prevalence of G6PD deficiency in Vatalia prajapathi community from western India." *Haematologia,* 31(1): 57-60.

Kaur, M., G.P. Das and I.C. Verma. 1997. "Sickle cell trait and disease among tribal communities in Orissa, Madhya Pradesh and Kerala." *Ind. J. Med. Res.*, 105: 111-116.

Mehta, B.C., A.J. Baxi, H.M. Bhatia, S.R. Joshi, V.B. Dave, P.N. Vaidya and J.C. Patel. 1971. "Glucose – 6-phosphate dehydrogenase deficiency in the Bhanusali community – A survey." *Ind. J. Med. Sci.,* 25:229-230.

Osaba, D., H.M. Dick, A. Voller, J.J. Goosen, T. Goosen and C. Draper. 1979. "Role of HLA complex in the antibody response to malaria under natural conditions." *Immunogenetics,* 8: 323-338.

Papiha, S.S., J. Wenzal, D.F. Roberts, Y.S. Rao and C.M. Habibullah. 1983. "HLA antigen frequency in the Koya tribe of Andhra Pradesh, India." *Am. J. Phys. Anthropol.,* 62: 147-150.

Potts W.K. and E.K. Wakeland. 1990. "The maintenance of MHC polymorphism." *Immunology Today*, 11: 39-42.

Pitchappan, R.M., K. Balakrishnan, V. Sudharsen, V. Brahmajothi, V. Mahendran, S. Amalraj, R. Santhakumari, K. Vijayakumar, P. Sivalingam and S. Ramasamy. 1997. "Sociobiology and HLA genetic polymorphism in hill tribes, the Irulas of Nilgiri hills and the Malayali of the shevory hills. South India." *Hum.Biol.,* 69: 59-74.

Rao, V.R. and H.M. Bhatia. 1988. "Quantitative, assessment of HbS in sickle cell hetreozygotes among some tribes in Maharastra." *Ind. J. Med. Res.,* 87: 257-261.

Rao, V.R. and A.C. Gorackshakar. 1990. "Sickle cell hemoglobin, beta thalasemia and G6PD deficiency in tribes of Maharastra, India." *Gene Geogr.,* 4: 131-134.

Ramasamy, S., K. Balakrishnan and R.M. Pitchappan 1994. "Prevalence of Sickle cell in Irula, Kurumba, Paniya and Mullukurumba tribes of Nilgiris (Tamil Nadu, India)." *Ind. J. Med. Res.,* 100: 242-245.

Raper, A.B. 1955. "Malaria and the sickling trait." *Br. Med. J.*, 1: 1186.

Reddy, P.H., M. Petron, P.A. Reddy, R.S. Tiwary and B. Modell 1955. "Hereditary anaemias and iron deficiency in a tribal; population (the Baiga) of the central India." *Eur. J. Haematol.*, 55: 103-109.

Riley, E.M., O. Olerup and M. Troye - Blomberg. 1991. "The immune recognition of Malaria antigens." *Parasitology Today*, 7: 5-11

Sanghvi, L.D., V. Balakrishnan and I. Karve (Eds.). 1981. *Biology of People of Tamil Nadu.* Calcutta: Indian Society of Human Genetics and the Anthropological Society of India. 75.

Selvakumar, A., B.K. Mohanraj, C. Damodharan, and P. Chandrasekaran 1987. Distribution of HLA antigens in Kotas & Badagas of the Nilgiri hills of South India. *Am. J. Phys. Anthropol.*, 74: 126-131.

Singh, K.S. 1993. "Peoples of India (1985-1992)." *Current Science*, 64: 1- 10.

Shankarkumar, U., K. Ghosh, S. Gupte, M.B. Mukerjee and D. Mohanty 1999. "Distribution of HLA antigen in Bhils and Pawars of Dhadgoan Maharastra, India." *J. Hum. Ecol.*, 10(3): 173-178.

Shankarkumar, U., S.V. Pednakar, S. Gupte, K. Ghosh and Mohanty D. 1999. HLA antigen distribution in Marathi speaking Hindu population from Mumbai, Maharastra India. *J. Hum. Ecol.*, 10(5-6): 367-372.

Troyo-Blomberg., O. Olerup, A. Larsson, K. Sjoberg, N. Perlmann, E. Riley, J.P. Lepers and P. Perlmann. 1991. "Failure to detect MHC Class II asssociation of human immune response induced to repeated malaria infection to *P. Falciparum* antigen Pf 155/ RESA." *Int.Immunol.*, 3: 1043-1051.

Vidhyarthi, L.P. 1983. Tribes of India. In: *Peoples of India - Some Genetic Aspects.* New Delhi: ICMR Publications., 85-103.

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