this figure is comparable to the findings of Nadas *et al.*(2) 7.5%, Keith, *et al.*(3) 9.9% and other Western workers(7). The relative rarity of coarctation of aorta among Indian population is also confirmed by other workers(5,8,9).

The observed female preponderance of ventricular septal defect is not compatible with the results of Bidwai *et al.*(5) and Rao and Reddi(8), who found a male predominance for the same. However, increased frequency of aortic stenosis and atrial septal defect among male patients is also supported by Jain *et al.*(6) and Rao and Reddi(8).

Our study suggests that a majority of congenital heart diseases in children may remain undetected unless specific efforts are made to diagnose them and that the prevalence of ventricular septal defect is the highest amongst school children.

REFERENCES

1. Rauh LW. The incidence of organic heart disease in school children. Am Heart J 1930, 18: 705-707.
2. Nadas AS, Fyler DC. Pediatric Cardiology, 3rd edn. Philadelphia, WB Saunders, 1973, pp 293-348 and 554-557.
3. Keith JD, Rowe RD, Vlad P. Heart Disease in Infancy and Childhood, 3rd edn. New York, Macmilan, 1978, pp 219 and 365-400.
4. Hoffman JIE, Christianson R. Congenital Heart Disease in a cohort of 19502 births with long term follow up. Am J Cardiol 1978, 42: 641-644.
5. Bidwai PS, Mahajan CM, Walia BNS, Berry JN. Congenital heart disease in childhood-A clinical study. Indian Pediatr 1971, 10: 691-694.
6. Jain KK, Sagar A, Beri S. Heart disease in children. Indian J Pediatr 1971, 30: 441-448.
7. Mitchell SC, Karones SB, Berendes BW. Congenital heart disease in 56109 births: Incidence and natural history. Circulation 1971, 43: 324-332.
8. Rao VS, Reddi YR. Profile of heart disease in children. Indian J Pediatr 1974, 41: 244-248.
9. Srivastava S, Tandon R. Congenital heart disease. *In:* Ahuja MMS. Progress in Clinical Medicine in India, 3rd series, New Delhi, Arnold-Heinemann, 1982, pp 196-200.
10. Mathur KS, Sapru BP. The etiology and incidence of heart disease–A changing pattern over the 15 years period 1947-1961. J Assoc Physicians India 1963, 11: 651-655.

Prenatal Diagnosis of Sickle Cell Anemia Using Polymerase Chain Reaction

M.P. Bankar
S.L. Kate
S.A. Ranade
R.J. Barnabas
G.D. Mokashi
M.A. Phadke
M.V. Hegde

Sickle cell mutation is one of the first reported single gene abnormality in the world, causing diseased condition in the homozygous state. Sickle cell hemoglobin is caused due to mutation in 6th codon of beta globin gene (a change from CTC to CAC in coding sequence of DNA).

WHO has reported an estimated 60×10^6 carriers in the world for hemoglobin S and 1,20,000 homozygotes are added every year in the world(1). In India, there are about

24,34,375 heterozygotes based on 1981 census data(2). In Maharashtra, there are two districts (Dhule and Chandrapur) having more than 5,000 estimated homozygotes.

Prevention of sickle cell disease by carrier detection and marriage counselling is relatively difficult. In majority of cases the couple at risk is detected only after the birth of a diseased child. Hence, a method is standardized to detect sickle cell mutation prenatally using polymerase chain reaction (PCR), at the molecular level *(Fig. 1)*.

Material and Methods

Two families, one from scheduled caste and one from scheduled tribe was known to be at risk for sickle cell disease. Parents were carriers for sickle cell hemoglobin. They had a child suffering from sickle cell disease. Ten ml venous blood was collected from each family member for DNA isolation. DNA was isolated by salting-out procedure(5). Fetal cells were obtained by CVB, for one patient and by amniocentesis in the other. DNA from the fetal cells was also isolated by the above procedure.

To detect the sickle cell mutation, 299 bp segment of the beta globin gene is amplified(6). PCR was performed on normal (AA), trait (AS) and homozygous (SS) DNA samples along with fetal DNA on Perkin and Elmer thermocycler with 94°C, 50°C and 72°C as denaturation, annealing and extension temperatures, respectively. So picomoles of each primer, 200 nanomoles of dNTP mixture, and 1 micgrogram or less of DNA were added in a 100 microliter of reaction mixture and was denatured at 95°C for 5 minutes. 2.5 units of Taq polymerase (Boehringer Mannheim) was added to the reaction mixture and 30 cycles were performed.

PCR product from each reaction is digested with 10 units of Mst II restriction enzyme for 1 hour at 37°C and analysed by agarose gel electrophoresis (1.5%), stained with ethidium bromide, visualized and photographed on a UV transilluminator (302 nm, Fotodyne). A marker (pBR322 Hinf I digest) was also electrophoresed alongwith the digested product.

Results

Mst II digestion products of normal, trait and homozygous for sickle cell hemoglobin revealed a pattern of 192 and 107 bp; 299, 192 and 107 bp; and 299 bp, respectively. The amplified and restriction digested samples of fetal DNA showed that one of them is heterozygous for sickle cell mutation while the other is normal for the same *(Fig. 2)*. Both were checked after delivery and found correct.

Discussion

PCR is a recently developed procedure for the rapid *in vitro* amplification of DNA sequences and it was a profound impact on various branches of biochemical sciences including the field of clinical diagnosis.

PCR has been successfully employed in diagnosis of infectious diseases, genetic disorders, cancer diagnostics, and to study evolutionary pattern(4). In the case of sickle cell hemoglobin the mutation in the 6th codon, eliminates the Mst II restriction site.

From the Tribal Health Research Project, Department of Pediatrics, B.J. Medical College, Pune 411 001 and Department of Biochemistry, University of Poona, Pune 411 007.

Reprint requests: Dr. (Mrs) Mrudula A. Phadke, Department of Pediatrics, B.J. Medical College and Sassoon General Hospital, Pune 411 001.

Received for publication: November 18, 1992; Accepted: February 18, 1993

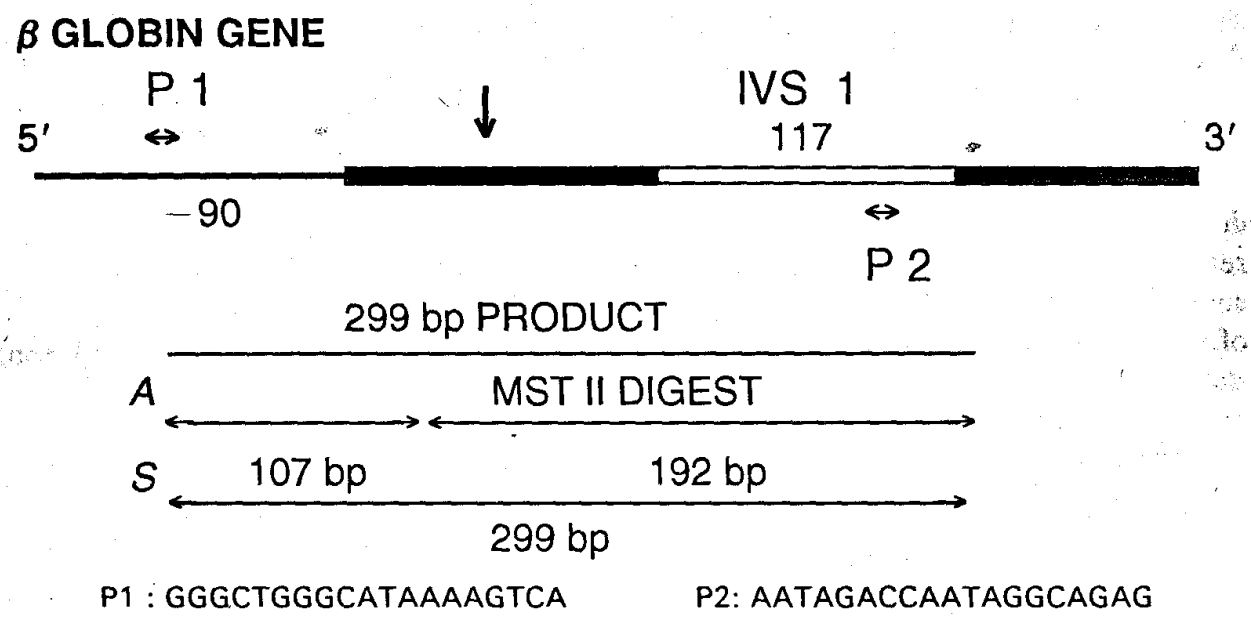

Fig. 1. PCR as carried out for diagnosis of sickle mutation. The region of B globin gene that is amplified is indicated. P_1 and P_2 are the primers selected for amplification cycles. The position of Mst II site change due to mutation is indicatedby an arrow. A - represents normal allele and S - represents sickle cell mutation.

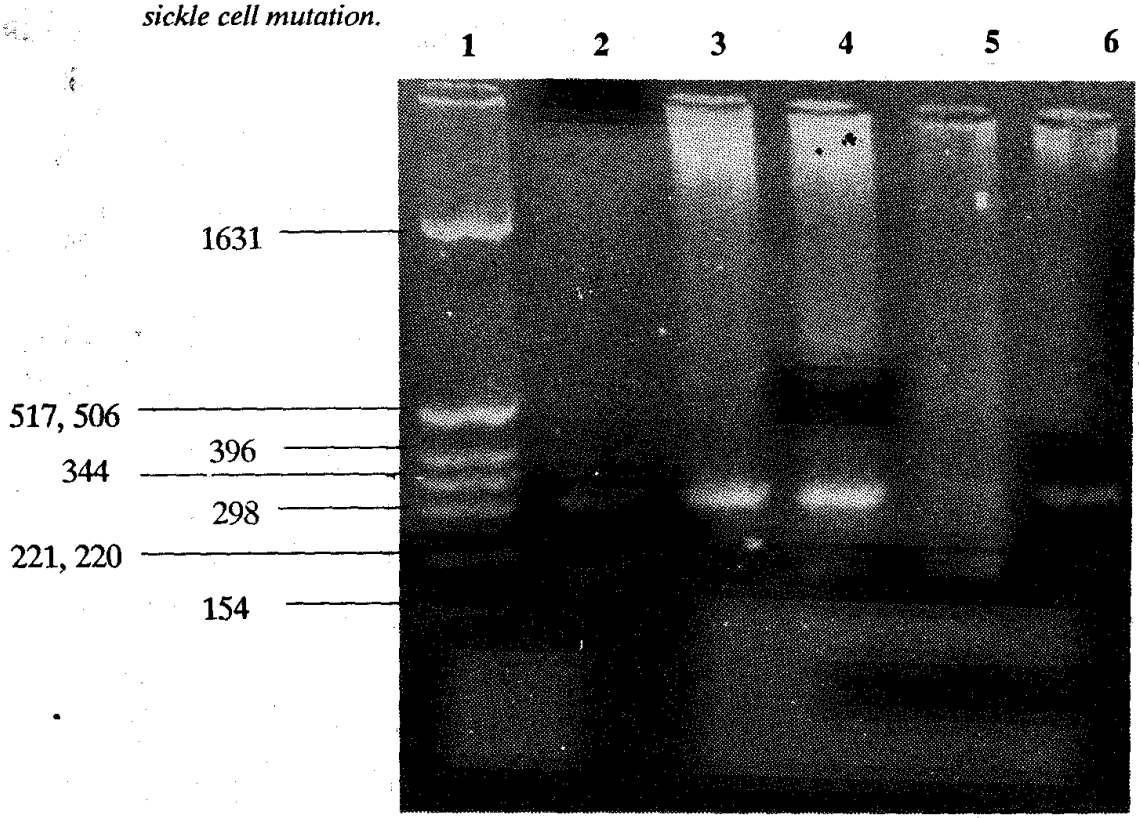

Fig. 2. Electrophoresis of the amplified DNA after Mst II digestion in 1.5% agarose gel in TEB buffer. (i) pBR Hinf I digest. Molecular weights are indicated in base pairs. (ii) Mst IIdigestion of amniotic fluid DNA. (iii) Mst II digestion of sickle cell anaemia (SS) 299 bp. (iv) Mst II digestion of sickel cell trait (AS) 299, 192 and 108 bp. (v) Mst II digestion of normal control (AA) 192 and 107 bp. (vi) Undigested amplified 299 by product.

Hence, PCR amplification followed by Mst II digestion confirms the sickle cell mutation, both in homozygous as well as heterozygous states.

Hence, using PCR technique and restriction digestion it is possible to diagnose antenatally single gene mutation. For this, no radioactive material or any external probe is used. The diagnosis is possible by the second day after the biopsy and it can be performed in the first trimester of pregnancy. Hence, medical termination of pregnancy if required, can be recommended. This technique (PCR), coupled with non-radioactive probing can also be effectively used for the prenatal diagnosis of thalassemia.

Acknowledgements

The authors thank Dr. K.N. Ganesh, National Chemical Laboratory, Pune, for providing synthetic oligonucleotide primers and Dr. D.A. Gadkari, National Institute of Virology, Pune, for use of their thermocycler.

REFERENCES

1. Community control of hereditary anemias. Memorandum from a WHO meeting. Bull WHO 1983, 61: 63-80.
2. Rao VR. Genetics and epidemiolgy of sickle cell anemia in India. ICMR Bull 1988, 18: 1-4.
3. Saiki RK, Scharf S, Faloona F, *et al.* Enzymatic amplification of beta globin genomic sequences and restriction site analysis of sickle cell anemia. Science 1985, 230: 1350-1354.
4. Gibbs RA. DNA amplification by polymerase chain reaction. Anal Chem 1990, 62: 1202-1214.
5. Miller SA, Dykes DD, Polensky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988, 16: 1215.
6. Chehab FF, Doherty M, Cai S, Kan YW, Kooper S, Rubin EM. Detection of sickle cell anemia and thalassemias. Nature 1987, 329: 294-295.

False Positive Widal Reaction in Malaria

J.R. Sharma
I.B. Parmar
S.J. Sharma
A. Kesavan

Both malaria and typhoid fever are endemic in Surat and the surrounding areas of South Gujarat. The diagnosis of malaria is clinical and confirmed by the presence of malarial parasite in the peripheral smear. Widal test inspite of its nonspecificity and unreliability, is still used as the gold standard for the diagnosis of typhoid fever(1). A positive widal reaction in malaria poses a diagnostic dilemma in the evaluation of a

From the Department of Pediatrics, Government Medical College, Surat, Gujarat.

Reprint requests: Dr. Jayendra R. Sharma, Assistant Professor of Pediatrics, B-7, Assistant Prof. Quarters, Government Medical College, Surat 395 001.

Received for publication: March 3, 1992; Accepted: April 21, 1993