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# A Sickle Cell Disease Carrier Family with a Pair of Dizygotic Twins from Kalahandi District of Western Orissa, India

**R. S. Balgir**

*Division of Human Genetics, Regional Medical Research Centre (ICMR), Opposite Kalinga Hospital, Chandrasekharpur, Bhubaneswar 751 023, Orissa, India*
*E-mail: balgirrs@yahoo.co.in*

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**ABSTRACT** The sickle cell disease is a genetically inherited commonly encountered hematological disorder that causes high degree of morbidity, mortality and fetal wastage. The suspected cases of hemoglobinopathies suffering from anemia are routinely referred from different peripheral Primary Health Centres and Hospitals in the state of Orissa (India) to our Centre for detailed investigations and genetic/marriage counselling. Of these, a scheduled caste (Domb) family with twin children was referred from Kalahandi district of the state and was studied in detail. A carrier couple of sickle cell disease had born twin offspring with both carrier as well as the homozygous sickle cell disease. It is a rare occasion when a single pregnancy carries either two or more distinct abnormal genotypes at a time in a womb in human beings. Dizygotic twins are genetically different and provide appraisal of the expression of different genotypes under the same environment.

## INTRODUCTION

Since the time of Sir Francis Galton who studied intelligence in twins in the nineteenth century to reveal the contribution of heredity, human twins have provided research inputs for the appraisal of nature-nurture controversy for determining the relative effects of genetics and environment. They provide the simplest and most powerful method for disentangling the influence of environmental and genetic factors on human characteristics. As the identical, monovular or monozygotic (MZ) twins are more similar with respect to same genotype or the same genes than the fraternal, binovular or dizygotic (DZ) twins, therefore, this is taken as evidence of a genetic influence on a particular characteristic. Identical twins are genetic duplicates. Both types of twins share common life experiences. Identical twins differ from fraternal twins in the number of genes they have in common. By studying identical and fraternal twins and their families, we can estimate how genes and environment interact to influence characters, strengths, vulnerabilities, and values.

Hemoglobinopathies are a group of hereditary disorders, highly prevalent in some population groups, and pose a major public health problem in the world. The sickle cell anemia especially affects 60-70 million people all over the world (Angastiniotis et al. 1995).

To the best of knowledge, no study has ever reported the occurrence of twins in sickle cell carrier parents. This study reports for the first time a rare dizygotic twin pair with sickle cell disorders born to scheduled caste (Domb) sickle cell disease carrier parents in Kalahandi district of Western Orissa, India.

## MATERIALS AND METHODS

The suspected cases of hemoglobinopathies suffering from anemia are routinely referred from different peripheral Primary Health Centres (PHCs) and Hospitals in the state of Orissa to our Centre for detailed investigations and genetic/marriage counselling. Out of these referral cases, a scheduled caste (Domb) family with twin children was referred to our Centre for detailed laboratory investigations. Background information for this family such as name, age, sex, caste, native place, reproductive history, family pedigree, and clinical signs and symptoms was also recorded. The index case and other available family members such as parents, brother/sister after taking informed/written consent were also subjected to clinical examination and laboratory investigations for confirmation of the diagnosis. They were also imparted genetic/marriage counselling accordingly as a measure to prevent further spread of the disease in the community.

2-3 ml intravenous blood samples were

collected using EDTA as anticoagulant by disposable syringes and needles from each individual under aseptic conditions after obtaining the informed consent. All the signs and symptoms related to sickle cell disease were recorded on the pre-designed proforma after clinical examination. Laboratory investigations were carried out following the standard procedures. Hematological parameters were studied by using an automated Blood Cell Counter (Model- MS4, Melet Schloesing Laboratories, France).

The sickling test was performed by using freshly prepared sodium metabisulphite solution as reducing agent for the presence or absence of sickle cell hemoglobin (Dacie and Lewis 1991). The routine hemoglobin lysate electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-Borate buffer at pH 8.9 and quantification of $A_2$ fraction of adult hemoglobin was done by elution method (Weatherall 1983); the value more than 3.5% of $A_2$ fraction of adult hemoglobin was taken as cut off point for determining the β-thalassemia trait. Estimation of fetal hemoglobin was done as described by Weatherall (1983). All the blood samples were further subjected to confirmation by doing the hemoglobin variant analysis (Bio Rad Laboratories, Pvt. Ltd., Australia).

## OBSERVATIONS AND RESULTS

Both parents (father and mother) were clinically examined by a medical doctor for signs and symptoms of sickle cell disease, irrespective of laboratory findings. They did not complain of any health problem. They were healthy young (husband aged 30 years and wife 25 years) couple having non-consanguineous marriage and belonged to scheduled caste (Domb) of Kalahandi district in Western Orissa. They had neither taken any blood transfusion nor suffered from jaundice or respiratory problems. Laboratory investigations showed the sickling test positive for both parents. Both parents had normal hematological (red cell) indices as well as red cell morphology. Alkaline (pH 8.9) electrophoresis of blood lysate on cellulose acetate membrane (CAM) showed both parents as the carriers of sickle cell disease, with other constituent hemoglobins ($A_2$ and fetal) falling in the normal range. The quantification of the sickle (HB S) bands revealed 34.4% and 26%, respectively for father and mother, the low percentage of HB S indicated the probability of interaction of sickle gene with alpha-thalassemia at least in the mother.

Out of a full-term son and daughter twin pair aged 3 years born to these parents, the elder daughter had usual fever, cough and cold occasionally in the winter season. She had no other complaints or complications, although her hemoglobin level was low (9.2 g/dl). Her hemograms or red cell indices were also low. Her sickling test was recorded to be positive. On electrophoresis, she was identified to be a case of sickle cell trait, with hemoglobin S constituting 24.9%; the low percentage of HB S suggests the involvement of alpha-thalassemia in the sickle cell disease.

The index case, the son who was born with low birth weight (as told by parents) having protruded abdomen, suffered from dactylitis in infancy. He started walking late indicating retarded growth and development, had cough and cold frequently, suffered from fever every month, and already had two times whole blood (200ml each time) transfusions after hospitalization with a gap of one year. At the time of presentation, he had recurrent fever, moderate anemia with splenomegaly (4 cm). All his hematological indices showed low values (Table1). The sickling test was found to be positive. The alkaline (pH 8.9) hemolysate electrophoresis on CAM revealed a thick band (83.5%) at the position of S band, trailing towards anode. His fetal hemoglobin was detected raised (13.7%) with normal hemoglobin $A_2$ level (Table 1). Thus, this proband was diagnosed as a case of sickle cell disease.

Since both the proband brother (sickle cell disease) and sister (sickle cell trait) had different sickle cell genotypes under similar environmental conditions with different clinical manifestations and hematological profile, they were probably dizygotic twins.

## DISCUSSION

The most striking rare finding in the present study is the carrier couple of sickle cell disease that had born twin offspring with both the carrier status as well as the homozygous sickle cell disease (Table 1). There was an equal probability in every singleton pregnancy that a carrier couple

**Table 1: A sickle cell disease carrier scheduled caste (Domb) family with twin offsprings from Kalahandi district of Orissa.**

| *Parameters* | *Parents* | | *Dizygotic Twins* | |
|---|---|---|---|---|
| | *Father* | *Mother* | *Daughter* | *Son** |
| Age (in years) | 25 | 20 | 3 | 3 |
| Sex | M | F | F | M |
| Hb (g/dl) | 12.5 | 11.2 | 9.2 | 7.6 |
| RBC ($x10^6$/ml) | 5.0 | 4.5 | 4.8 | 3.5 |
| HCT (%) | 38.7 | 36.7 | 30.3 | 24.2 |
| MVC (fl) | 77.8 | 82.3 | 63.2 | 69.2 |
| MCH (pg) | 25.1 | 25.1 | 19.1 | 21.7 |
| MCHC (g/dl) | 32.2 | 30.5 | 30.3 | 31.4 |
| RDW (%) | 9.0 | 7.6 | 10.2 | 9.7 |
| WBC ($x10^3$/ml) | 6.8 | 8.4 | 10.7 | 9.5 |
| Sickling Test | +ve | +ve | +ve | +ve |
| Electrophoresis (pH 8.9) | $A+S+A_2$ | $A+S+A_2$ | $A+S+A_2$ | $S+F+A_2$ |
| Hb $A_2$ (%) | 2.5 | 2.0 | 2.1 | 2.8 |
| Hb F (%) | 0.2 | 0.6 | 0.3 | 13.7 |
| Hb S (%) | 34.4 | 26.0 | 24.9 | 83.5 |
| Hb A (%) | 62.9 | 71.4 | 72.7 | - |
| Diagnosis | Sickle Cell Trait | Sickle Cell Trait | Sickle Cell Trait | Sickle Cell Disease |

* Proband

of sickle cell disease would either bear normal, carrier or sickle cell disease offspring, but not the two of these conditions (genotypes) together. It is a rare occasion when a single pregnancy carries either two or more distinct abnormal genotypes at a time in a womb in human beings. The present study reports for the first time the occurrence of dizygotic twins in a singleton pregnancy in the sickle cell carrier parents from Kalahandi district of Orissa, India.

It is the Nature rather than the nurture. Fraternal or dizygotic twins share 50% of their genes. Dizygotic twins with different sickle cell genotypes under similar environmental conditions manifest different clinical and hematological picture. In other words, dizygotic twins are genetically different and, thus, provide means of appraisal of the expression of different genotypes under the same environment. The present study points to the above ideological and conceptual debate.

Tests for prenatal diagnosis such as amniocentesis, cord blood or chorionic villi sampling, fetal biopsy, non-invasive technology, etc. already exist to check a fetus for sickle cell during pregnancy. But some couples have strong personal objections to abortion when a pregnancy is already established. Pre-implantation genetic diagnosis (PGD) could provide couples with another way to avoid passing on sickle cell disease. A couple who is carrier of sickle cell disease has twin babies who are completely free of the sickle cell gene with the application of a technique called pre-implantation genetic diagnosis (PGD), in which fertilized embryos are tested for the disorder before implantation in the woman's uterus (Xu et al. 1999). Further refinement in this technology will become a powerful diagnostic tool in the assistive reproductive technology (ART) in the near future.

Individuals with the sickle cell disease have recurring episodes of intense pain; an increased susceptibility to infections, strokes and organ failure; and a lowered life expectancy. It is very imperative that predictive or preventive genetics can play a significant role in mitigating the sufferings of the affected persons. Further molecular studies are called for in such type of dizygotic twins that may provide new insight into this common genetic and health problem in India.

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