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ORIGINAL ARTICLE

Population and Public Health Implications of Child Health and Reproductive Outcomes Among Carrier Couples of Sickle Cell Disorders in Madhya Pradesh, Central India

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ABSTRACT

Background: Sickle cell disease is a major genetic and public health challenge in India. Adequate studies on clinico-hematological aspects of disorders are available, however there are few studies on the public health and reproductive outcomes among sickle cell carrier couples.

Methods: A total of 383 couples including their offspring with at least one case of sickle cell disorder referred to a testing center from a tertiary hospital from March 2010 to February 2013 were consecutively studied as matched case controls.

Results: Out of 383 couples, 200 were found normal and 183 had different sickle cell disorders. Carrier couples of sickle cell disease had significantly higher fertility (mean number of conceptions, i.e. 3.153 versus 1.480) and higher below 10 year mortality (11% versus 2.7%) and lower surviving offspring (877.4 versus 970.6) than of controls. Neonatal and infant mortality was doubled (34.3 versus 14.7) and three-fold higher (44.1 versus 14.7), respectively in carriers of disease per 1000 live-births compared to controls. Couples of AS/SS genotype showed high neonatal, infant, below 10 year mortality (214.3 each) and low surviving offspring (785.7 per 1000 live-births).

Conclusions and Global Health Implications: Sickle cell carrier couples are increasing in both trait and disease offspring (surviving: 56.7% against 43.3% normals). This increased production of carrier and disease offspring leads to increased morbidity, neonatal/infant and childhood mortality, and adversely affects the survival fitness.

Key words: Sickle cell disease • β-thalassemia major • Fertility • Reproductive loss • Neonatal mortality • Infant mortality • Central India

Introduction

The sickle cell disorders are one of the major genetic and public health challenges in India.[1, 2] Anemia is a major morbidity among people with sickle cell disease. Anemia in pregnancy is an important cause of maternal complications, maternal and fetal morbidity and mortality in almost all the developing countries of the world including India.[3, 4] Patients suffering from sickle cell disease are generally anemic and are susceptible to infections that cause aggravation and severe clinical manifestations leading to early death. Affected infants with sickle cell disease may present with dactylitis, fever and overwhelming sepsis, chronic hemolytic anemia, jaundice, episodic vaso-occlusive crises, hyposplenism, periodic splenic sequestration (which can be life threatening in a small child) and bone marrow sepsis.[5-7] Inadequate availability of oxygen to fetus also leads to abortion, miscarriage or stillbirth. Genetically vulnerable groups include: infants, growing children, adolescents, pregnant women and a large number of ignorant people. Inherited disorders of hemoglobin cause high degree of hemolytic anemia, clinical jaundice, frequent infections, painful crises, splenomegaly, etc. and are responsible for the high infant morbidity, mortality and fetal loss in populations of under developed and developing tropical countries of the world.[8, 9]

The purpose of this study was to screen and identify the couples with and without sickle cell disorders and to compare their reproductive outcomes with regards to abortions, miscarriages or stillbirths, neonatal mortality, infant mortality, and childhood mortality. The goal is to investigate whether carrier couples of sickle cell disorders independently contribute more towards reproductive loss as a result of hemolytic anemia in the families and consequently to the population than the couples without these disorders. It also investigated whether the identified difference are due to confounding non-dependable variables such as birth asphyxia, pre-eclamptic toxemia, puerperal sepsis, prematurity, low birth-weight babies, maternal malnutrition, serious malarial or other urino-genital tract infections, diarrhea, immunological incompatibility (such as ABO and Rhesus blood groups) between mother and fetus (HDN), congenital anomalies or hereditary hemolytic disorders such as glucose-6-phosphate dehydrogenase (G6PD) deficiency, and physical injuries during intra-uterine period/delivery. Keeping the non-dependable confounding variables almost similar, being taken from the same source for both these groups, the difference in two groups, if any amounts to reproductive outcome of the sickle cell disorders specifically of the homozygous sickle cell disease.

Madhya Pradesh state is predominantly poor, prevalent low-birth-weight babies with undernourishment and anemia, poor child feeding practices, food insecurity, vulnerability to infectious environment, limited access to basic health care services, and overwhelming neonatal/infant mortality. The early identification and intervention of infants with sickle cell disease has shown markedly reduced morbidity and mortality elsewhere.[3] The present study will be of immense interest to the global health community because along with the screening and identification of infants with sickle cell disorders, the exploration of reproductive outcome in carrier couples of sickle cell disease has also been investigated. This study is among the first to explore the reproductive loss due to sickle cell disorders in terms of abortions, miscarriages or stillbirths, neonatal mortality, infant mortality, and childhood mortality in the vulnerable couples from central India where a huge population is afflicted with sickle cell disease disability.[10, 11]

Methods

A total of 383 suspected couples including their offspring with at least one suspected/ confirmed case of anemia/sickle cell disorders routinely referred by the experts (in Gynecology, Pediatrics, and Blood Bank) for investigations/confirmation of diagnosis, attending Netaji Subhash Chandra Bose Medical College & Hospital, Jabalpur, Madhya Pradesh in Central India were included in the study. Ethical clearance was obtained from the Human Ethical Committee of Regional Medical Research Centre for Tribals, Jabalpur, India.

Confirmed carrier couples of sickle cell disorders formed our study group and the negatives (normal and without sickle cell disorders), free of

hematological disorders/anemia, after rigorous scrutiny, were taken as controls. Cases suffering from other hemoglobinopathies and genetic abnormalities and with iron deficiency anemia, hematological disorders, malaria, accidental or induced abortion (dependable variables) were excluded from the study. All the non-genetic confounding factors more or less were similar for both groups (matched case controls), being taken from the same population source. Detailed reproductive history of each couple was recorded retrospectively like total number of conceptions, abortions, miscarriages or still-births, live-births, surviving children, neonatal or infant deaths, etc. Table 1 provides a breakdown of the couples investigated in the study. Out of 383 couples investigated, 200 were found normal and 183 couples had different sickle cell disorders. A total of 1397 persons (703 males and 694 females) were investigated for sickle cell disorders including the controls.

Intravenous 2-3ml blood was taken under aseptical conditions from each individual after taking informed/written consent for screening of sickle cell disorders. Hematological parameters were studied using an automated Blood Cell Counter (Model-$MS_5$9, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France). Sickling test was performed using 2% freshly prepared sodium metabisulphite solution as reducing agent for the presence or absence of sickle cell hemoglobin.[12] The routine hemoglobin (Hb) lysate electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-Borate buffer at pH 8.6 and quantification of A_2 fraction of adult hemoglobin was done by elution method.[12, 13] The value of 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.[13]

The diagnosis of sickle cell-β-thalassemia was based on the findings of hemoglobin A, F, S and A_2 on electrophoresis under alkaline pH, elevated (>3.5%) A_2 levels.[2] All the doubtful cases were further subjected to hemoglobin variant analysis for detection of any discrepancy (Bio-Rad Diagnostics, Hercules California, USA). Data results were given to parents for treatment and further clinical management by the concerned referring doctor. All the carrier/affected persons were given genetic/marriage counseling.

Table 1 Distribution and genotypes of couples with and without different sickle cell disorders

No. of Couples (N)	Genotypes of Couples (Diagnosis)	Interpretation of Genotypes
65	AA/AS	Normal husband and sickle cell trait wife or normal wife and sickle cell trait husband
18	AA/SS	Normal husband and sickle cell disease wife or vice versa
72	AS/AS	Both husband and wife are carrier for sickle cell disease
9	AS/SS	One partner is carrier for sickle cell disease and other partner is suffering from sickle cell disease
15	AS/β-Thalassemia Trait	One partner being carrier for sickle cell disease and the counterpart is β-thalassemia trait (or carrier of thalassemia major)
4	AS/Sickle cell-β-Thalassemia	One partner being carrier of sickle cell disease and the other partner is sickle cell-β-thalassemia (having compound disease, i.e. sickle cell disease and β-thalassemia)
183	Sickle Cell Disorders (All above combined)	All the above diagnostic categories (genotypes) combined together except the normal controls
200	AA/AA (Normal)	Normal husband and normal wife (control)

Results

Conceptions. It was observed that the average number of conceptions per couple was higher in different sickle cell disorders [AS/AS (3.153); AA/AS (2.000), AA/SS (1.667); AS/SS (1.778); AS/β-thal (3.333); AS/S-β-thal (2.250); and β-thal/S-β-thal (2.250)] than in normal controls (1.480).

Abortions. Carrier couples of sickle cell disease (Table 2) had more abortions (7.0%) than in the controls (4%). Similar results were available for couples of AA/AS and AA/SS genotypes. The frequency of abortions was lower in AS/sickle cell-β-thalassemia (0.0%) and AS/SS (0.0%) couples than in the normal (4%) controls (Table 2). The number of abortions per 1000 live-births was almost doubled in carrier couples of sickle cell disease (78.4), followed by AA/AS (90.1) and AA/SS couples (142.8) than in normal controls (44.1) (Table 3).

Still Births. Couples of AA/SS genotypes had significantly ($p<0.001$) higher number of still-births (20.0%) than the controls (3.7%). Almost similar results were obtained in couples of carriers of sickle cell disease and AS/S-β-thalassemia (Table 2). However, the number of still-births per 1000 live-births was higher in couples of sickle cell disorders, AS/SS, AA/AS, AS/S-β-thalassemia, and AA/SS than the controls (40.4) (Table 3).

Neonatal Deaths. The number of neonatal deaths was significantly higher in couples of AS/sickle cell-β-thalassemia ($p<0.05$) and also significantly higher in AS/SS couples ($p<0.001$) compared to controls (Table 2). However, overall couples of sickle cell disorders were not significantly ($>0.05p<0.10$) different from the normal couples with respect to neonatal mortality. Neonatal deaths in carrier couples of sickle cell disorders constituted 85% of the infant deaths and 42% of the <10 years deaths (Table 2). Neonatal mortality per 1000 live-births was almost doubled in carriers of sickle cell disease (34.3), and higher in couples of AS/β-thalassemia trait, AA/AS, AA/SS, AS/S-β-thalassemia, and AS/SS than in controls (14.7) (Table 3).

Infant Mortality. Couples of sickle cell disorders as a whole and AS/sickle cell-β-thalassemia showed significantly ($p<0.05$) higher infant mortality and still higher in AS/SS couples ($p<0.001$) than in the controls (Table 2). However, the carrier couples of sickle cell disease did not show significant ($<0.10p>0.05$) difference from normal controls for infant mortality. Infant mortality contributed 50% of <10 year deaths in carrier couples of sickle cell disorders (Table 2). Infant mortality per 1000 live-births was three-folds higher (Table 3) in carriers of sickle cell disease (44.1), and higher in couples of AS/β-thalassemia trait, AA/AS, AA/SS, AS/S-β-thalassemia, and AS/SS than in controls (14.7). The carrier couples of sickle cell disease, AS/SS, and the couples with sickle cell disorders as a whole showed highly significant higher mortality under 10 years of age ($p<0.001$) than the normal controls (Table 2). Similar results were noted for couples of AS/β-thalassemia trait and AS/S-β-thalassemia compared to controls. Similar results were obtained in carrier couples of sickle cell disorders per 1000 live-births for mortality <10 years of age (Table 3).

Live Births. Couples of AA/SS genotypes had significantly ($p<0.02$) lower number of live-births (70.0%), followed by AA/AS couples ($p<0.05$) than the controls (91.9%) (Table 2). However, there was significantly higher number of live-births in couples of AA/AS ($p<0.05$), and AA/SS ($p<0.001$) than the controls. However, over all couples of sickle cell disorders did not show significantly higher number of live-births ($<0.10p>0.05$). There was significantly higher number of surviving offspring in couples of AA/AS, AA/SS, and sickle cell disorders ($p<0.001$) than the controls. The sickle cell disease carriers ($p<0.01$) and AS/SS couples ($p<0.05$) also showed significantly higher number of surviving offspring than the controls (Table 2). Couples of AS/SS genotype had the lowest number of surviving children per 1000 live-births (Table 3).

Discussion

Findings from this study show many practical aspects of couples with recessively inherited hemolytic disorders such as sickle cell disease in Central India. This study supports the contention that hereditary factors in the carrier couples, apart from concomitant nongenetic confounding factors, are responsible for

Table 2 Comparison of reproductive history of carrier couples with and without different sickle cell disorders

Genotypes of Couples (Diagnosis)	No. of Couples N	Total No. of Conceptions	No. of Livebirths n	%	No. of Abortions n	%	No. of Stillbirths n	%	No. of Neonatal Deaths ● n	%	No. of <1Year Deaths ■ n	%	No. of <10Years Deaths n	%	No. of Surviving Offspring n	%
AA/AS	65	130	111	85.4[a]	10	7.7	9	6.9	4	3.1	4	3.1	6	4.6	105	80.8[d]
AA/SS	18	30	21	70.0[b]	3	10.0	6	20.0[d]	1	3.3	1	3.3	1	3.3	20	66.7[d]
AS/AS	72	227	204	89.9	16	7.0	7	3.1	7	3.1	9	4.0	25	11.0[d]	179	78.8[c]
AS/SS	9	16	14	87.5	0	0.0	1	6.2	3	18.7[d]	3	18.7[d]	3	18.7[d]	11	68.7[b]
AS/β-Thal.Trait	15	50	49	98.0	1	2.0	0	0.0	1	2.0	2	4.0	4	8.0	45	90.0
AS/S-β-Thalassemia	4	9	8	88.9	0	0.0	1	11.1	1	11.1[a]	1	11.1[a]	1	11.1	7	77.8
Sickle Cell Disorders (combined above all)	183	462	407	88.1	30	6.5	24	5.2	17	3.7	20	4.3[a]	40	8.6[d]	367	79.4[d]
AA/AA (Normal)	200	296	272	91.9	12	4.0	11	3.7	4	1.3	4	1.3	8	2.7	264	89.2

Difference between diagnostic categories and normal controls is statistically significant at:
a=p<0.05; b=p<0.02; c=p<0.01; d=p<0.001 level.

● Birth to 28 days (Neonatal Mortality). ■ Birth to 365 days or within 1 year (Infant Mortality).

N= Number of couples; n= Number of observations; AA = Normal Adult Hemoglobin; AS = Sickle Cell Trait; SS = Sickle Cell Disease; β-Thal.Trait = Beta-Thalassemia Trait; S-β-Thal. = Sickle Cell-β-Thalassemia.

Table 3 Comparison of reproductive history (figures are 1000 live-births) of carrier couples with and without different sickle cell disorders

Genotypes of Couples (Diagnosis)	No. of Couples	No. of Conceptions	No. of Livebirths	No. of Abortions	No. of Stillbirths	No. of Neonatal Deaths ●	<1Year Deaths ■	<10Years Deaths	Surviving Offspring
AA/AS	65	130	111	90.1	81.1	36.0	36.0	54.0	945.9
AA/SS	18	30	21	142.8	285.7	47.6	47.6	47.6	952.4
AS/AS	72	227	204	78.4	34.3	34.3	44.1	122.5	877.4
AS/SS	9	16	14	0.0	71.4	214.3	214.3	214.3	785.7
AS/β-Thal.Trait	15	50	49	20.4	0.0	20.4	40.8	81.6	918.4
AS/S-β-Thal.	4	9	8	0.0	125.0	125.0	125.0	125.0	875.0
Sickle Cell Disorders (combined above all)	183	462	407	37.7	59.0	41.8	49.1	98.3	901.7
AA/AA (Normal)	200	296	272	44.1	40.4	14.7	14.7	29.4	970.6

Sickle cell disorders.

● Birth to 28 days (Neonatal Mortality). ■ Birth to 365 days or within 1 year (Infant Mortality).

the high reproductive loss in the form of abortions, still-births, neonatal and infant mortality, and mortality <10 years of age in India (Table 2). This is an outcome of nonviable homozygosity of the recessively inherited genetic disorders such as sickle cell disease due to inbreeding that is inadvertently taking place in the vulnerable communities of the region that enhances morbidity and neonatal/infant mortality. This is the 1st study carried out taking into consideration these causative aspects of high mortality in the state of Madhya Pradesh, India. These results are consistent with the similar findings reported from Odisha state[8] in eastern coast of India.

These findings have also been supported by the high neonatal mortality rate which was 44 and 33 for Madhya Pradesh and India, respectively in the year 2010. Neonatal mortality was found almost doubled in carriers of sickle cell disease (34.3) than in controls (14.7) per 1000 live-births in the present study (Table 3). Similarly, the infant mortality rate of 62 was the highest for Madhya Pradesh as compared to 47 for India in the year 2011. This infant mortality rate has declined in Madhya Pradesh to 56 in the year 2012.[14] The overall neonatal and infant mortality was recorded for carrier couples of sickle cell disorders to be 41.8 and 49.1, respectively per 1000 live-births against controls (14.7 for each category) in the present study.

There are very few studies available on the subject of fetal loss in India. Earlier in the state of Odisha, high neonatal (48.3) and infant (75.9) mortality per 1000 live-births was reported in carrier couples of sickle cell disease.[8] The findings of the present study are not at variance from that of Odisha with respect to neonatal (41.8) and infant (49.1) mortality in Madhya Pradesh. This consistency of findings shows almost similar pattern of consanguinity or inbreeding in Odisha in the communities possessing recessively inherited genetic characters such as sickle cell disease,[15] leading to increased homozygosity and reproductive loss, that is, abortions, still-births, neonatal and infant mortality or childhood mortality affecting the population fitness.

Looking at the overall scenario of reproductive loss and surviving offspring in different carrier couples of sickle cell disorders, the number of normal children born to carrier couples was lower (159/367; 43.3%) than the inflicted children (208/367; 56.7%), indicating the progressive increase of inflicted offspring in these families. This trend shows the lower fitness of the carrier couples or affected families, and, consequently, of the vulnerable population(s).

It has been observed that enhancement of the institutional delivery accompanied by quality public healthcare services provide added advantage to reproductive outcome, whereas, the widespread undernourishment and anemia among pregnant women lead to under-weight children. Moreover, lack of public health facilities to cope with specialized newborn care coupled with sickle cell disorders bring adverse reproductive outcomes. It was envisaged to bring awareness among these couples through genetic/marriage counseling about the deleterious genetic disorders and their causal effects on health. Their eradication is necessary because they are not curable but preventable through carrier detection, prenatal diagnosis and, education and genetic counseling. This has ample implications in those families, communities and tropical countries where sickle cell disease is widely prevalent and is a major cause of high morbidity and mortality. In order to bring the reduction/prevention and control of sickle cell disorders in affected families, all the referred and affected families were given genetic/marriage counseling to prevent the birth of an abnormal child in their families[16, 17] for the betterment of future generations.

Conclusions and Global Health Implications

The increased production of inflicted trait and disease surviving offspring (56.7%) than the normal children (43.3%), leads to increased morbidity and mortality, and may be contributing towards increased neonatal/infant mortality in Madhya Pradesh. This is among the first attempts to report hereditary causes, apart from other concomitant non-genetic factors, responsible for the high neonatal/infant mortality (reproductive loss) in this population. Further, the progeny of sickle cell disease couples may be contributing to the disproportionate rate of neonatal/infant mortality in Madhya Pradesh. Genetic pre-marriage counseling is

highly recommended in both affected and unaffected couples/families to ameliorate potential burdens to individuals and families.

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References

1. Balgir RS. Epidemiology, population health genetics and phenotypic diversity of sickle cell disease in India. Internet Journal of Biological Anthropology. 2007; 1(2):1-26.
2. Balgir RS. Phenotypic diversity of sickle cell disorders with special emphasis on public health genetics in India. Current Science. 2010; 98(8):1096-1102.
3. Balgir RS. Hematological profile of pregnant women with carrier status of hemoglobin disorders in coastal Odisha, India. International Journal of Child Health and Human Development. 2011; 4(3): 325-332.
4. Balgir RS. A cross-sectional study of hemoglobin disorders in pregnant women attending two urban hospitals in eastern coast of Odisha, India. Online Journal of Health and Allied Sciences. 2013; 12(4):5.
5. Kar BC, Satapathy RK, Kulozik AE, Kulozik M, Sirr S, Serjeant BE. Sickle cell disease in Orissa state, India. Lancet. 1986; ii(8517):1198-1201.
6. Kar BC. Sickle cell disease in India. Journal of Association Physicians of India. 1991; 39(12): 954-960.
7. Balgir RS. Clinical genetics and hematological profile of sickle cell cases in twenty families of Orissa. Indian Journal of Hematology and Blood Transfusion. 2006; 22(1): 45-52.
8. Balgir RS. Infant mortality and reproductive wastage associated with different genotypes of haemoglobinopathies in Orissa, India. Annals of Human Biology. 2007; 34(1): 16-25.
9. Balgir RS. Reproductive wastage in carrier couples of hemoglobinopathies: experiences from a retrospective study in Madhya Pradesh, India. International Journal of Child Health and Human Development. 2013; 6(2): 235-242.
10. Balgir RS. Public health challenges of sickle cell disorders, β-thalassemia syndrome and G6PD deficiency in scheduled caste and scheduled tribe communities of central India. International Public Health Journal. 2011; 3(3): 307-318.
11. Balgir RS. Spectrum of hemoglobinopathies and evaluation of prevalence of beta-thalassemia trait in the tribal land of Middle India. International Public Health Journal. 2013; 5(2): 165-177.
12. Dacie JV, Lewis SM. Practical Hematology. 7th Edn. Churchill Livingstone, Edinburgh. 1991; 227-258.
13. Weatherall DJ. The Thalassemias. In: Methods in Hematology. Vol. 6. Churchill Livingstone, New York. 1983; 1-53.
14. James KS. Recent shifts in infant mortality in India: An exploration. Economic and Political Weekly. 2014; XLIX (3):14-17.
15. Balgir RS. Sickle cell disease and community health issues in Orissa. Indian Practitioner 1993; 46(6):427-431.
16. Balgir RS. Prevention of hereditary disorders in India: sickle cell disease, β-thalassemia and G6PD deficiency (in English & Oriya). Regional Medical Research Centre (Indian Council of Medical Research), Bhubaneswar. 2001; 1-12.
17. Balgir RS. Challenges of imparting IEC for prevention of hereditary sickle cell disorders, β- thalassemia syndrome and G6PD deficiency in India. Tribal Health Bulletin 2007; 13(1&2): 14-22.