

Hematological-genetics, Reproductive Health and Family Welfare in Madhya Pradesh, India

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ABSTRACT

Introduction: The sickle cell disease is a major genetic and public health challenge in Central India. In view of dubious credit of the highest infant mortality rate in Madhya Pradesh (62 as against 47 per 1000 live-births for India in 2011). **Aim:** It was presumed that carrier couples of sickle cell disorders might be one of the contributing factors to mortality. **Materials and Methods:** A total of 383 couples including their offspring with at least one affected and/or suspected case of sickle cell disorder referred to our Centre from NSCB Medical College & Hospital, Jabalpur during March 2010 to February 2013 were consecutively studied as matched case controls. **Results and Discussion:** Out of 383 couples, 200 were found normal and 183 couples had different sickle cell disorders. Sickle cell carrier couples had statistically significantly more relative fertility (mean number of conceptions, i.e. 3.153 versus 1.480) and more under 10 year mortality (11% versus 2.7%) and low surviving offspring (877.4 versus 970.6) than of normal controls. Neonatal and infant mortality per 1000 live-births was doubled (34.3 versus 14.7) and three-fold higher (44.1 versus 14.7), respectively in carriers of sickle cell disease than of controls. Couples with AS versus SS genotype showed high occurrence of neonatal, infant, under 10 year mortality each 214.3, respectively and low surviving offspring (785.7) per thousand live-births. **Conclusions:** This study indicated that afflicted couples (56.7%) of these hereditary disorders are increasing the defective (trait and disease) against 43.3% (controls) normals. This increased production of defective offspring leads to increased morbidity and mortality and might be contributing towards increased neonatal/infant mortality in Madhya Pradesh. As a preventive measure, affected families were imparted genetic/marriage counseling.

Key words: High fertility, India, infant mortality, Madhya Pradesh, neonatal mortality, reproductive wastage, sickle cell disease, β -thalassemia major

INTRODUCTION

The sickle cell disorders are one of the major genetic and public health challenges in India.^[1,2] Anemia in pregnancy is emerging as one of the most important causes of maternal complications, maternal and fetal morbidity and mortality in almost all the developing countries of the world including India.^[3] Patients suffering from sickle cell disease disability are generally anemic and are susceptible to infections that cause aggravation and severity of 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.^[4-6] Inadequate availability of oxygen to fetus also leads to abortion, miscarriage, or stillbirth. The genetic victims include infants, growing children, adolescent girls, pregnant women, and a large number of ignorant people. Inherited disorders of hemoglobin (Hb) 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 wastage in India.^[7,8]

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The primary purpose of screening is the identification of infants with sickle cell disease for whom early intervention has shown markedly reduced morbidity and mortality.^[3] A great deal of literature is available regarding the clinical and hematological aspects of these disorders, but the details regarding the reproductive outcome in affected couples are scanty in India. In view of credit for the highest infant mortality rate (IMR) in the state of Madhya Pradesh (62 per thousand live births in 2011) in comparison to other states and the average of 47 for India, and the high prevalence of hemoglobinopathies in the state,^[9] it was presumed that hemoglobinopathies, especially the sickle cell disorders, might be one of the significantly contributing factors for neonatal/infant mortality in carrier couples in Madhya Pradesh, India.

MATERIALS AND METHODS

A total of 383 suspected couples including their offspring with at least one suspected/confirmed case of anemia/hemoglobinopathies routinely referred by the experts (in gynecology, pediatrics, and blood bank) for investigations/confirmation of diagnosis, attending Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur, Madhya Pradesh in Central India were included in the study. The ethical clearance was obtained from the Human Ethical Committee of Indian Council of Medical Research-National Institute for Research in Tribal Health, Jabalpur.

Confirmed cases of sickle cell disorders formed our study group and the negatives, free of hematological disorders/anemia, after rigorous scrutiny, were taken as a control group. The cases, suffering from other hemoglobinopathies and genetic abnormalities, were excluded from the study. Those cases with iron deficiency anemia, other hematological disorders, malaria, accidental, or induced abortion, were also 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 such as total number of conceptions, abortions, miscarriages or stillbirths, live births, surviving children, and infant or neonatal deaths.

In this paper, genotypes of couples such as AA × AA stand for normal husband and normal wife (control); AA × AS denotes for normal husband and sickle cell trait wife or normal wife and sickle cell trait husband; AA × SS denotes for normal husband and sickle cell disease wife or vice versa; AS × AS denotes that both husband and wife are carrier for sickle cell disease; AS × SS means that one partner is carrier for sickle cell disease and another partner is suffering from sickle cell disease; AS × β-thal. trait stands for one partner being carrier for sickle cell disease and the another counterpart is beta-thalassemia trait (or carrier thalassemia major); the

genotype AS × S-β-thal. stands for one partner being carrier of sickle cell disease and the another partner is sickle cell-beta-thalassemia (having compound disease, i.e., sickle cell and beta-thalassemia); and sickle cell disorders mean here all the above diagnostic categories (genotypes) combined except the normal controls. A total of 1397 persons (703 males and 694 females) were investigated for sickle cell disorders including the controls. Out of 383 couples investigated, 200 were found normal and 183 couples had different sickle cell Hb disorders.

Intravenous 2–3 ml blood was taken under aseptic conditions from each individual after taking informed/written consent for screening of hemoglobinopathies. Hematological parameters were studied using an automated blood cell counter (Model-MS₃9, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France). Laboratory investigations were carried out following the standard procedures after cross-checking for quality control from time to time. The sickling test was performed using 2% freshly prepared sodium metabisulfite solution as a reducing agent for the presence or absence of sickle cell Hb.^[10] The routine Hb lysate electrophoresis was carried out on cellulose acetate membrane in Tris-ethylenediaminetetraacetic acid-borate buffer at pH 8.9 and quantification of A₂ fraction of adult Hb was done by elution method.^[10,11] The value more than 3.5% of A₂ fraction of adult Hb was taken as cutoff point for determining the β-thalassemia trait. Those individuals having the very high Hb A₂ value, i.e. more than 10% were suspected to have Hb A₂ plus Hb E; and the test was confirmed by the investigations of other family members. Estimation of fetal Hb was done according to technique described by Weatherall.^[11]

The diagnosis of sickle cell-β-thalassemia was based on the findings of Hb A, F, S, and A₂ on electrophoresis under alkaline pH, elevated HbA₂ levels (>3.5%). All the doubtful cases were further subjected to Hb variant analysis for detecting any discrepancy (made for 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 carriers/affected persons were imparted genetic/marriage counseling. Data collected were analyzed as per the standard statistical methods such as Chi-square test and calculation of reproductive wastage (abortions, stillbirths, neonatal mortality, and infant mortality) in percentages and also per thousand live births.

RESULTS

A total of 383 couples were referred during the period from March 2010 to February 2013. Out of 383 couples, 200 were found normal and 183 couples had different sickle

cell Hb disorders. A total of 1397 persons (703 males and 694 females) were investigated for sickle cell disorders including the controls [Table 1].

It was observed that the average number of conceptions per couple was considerably higher in couples with different sickle cell disorders: AS versus AS (3.153), AA versus AS (2.000), and AS versus β -thalassemia trait (3.333) than the normal controls (1.480). The frequency of abortions was lower in couples with AS versus sickle cell- β -thalassemia and in AS versus SS couples than the normal controls [Table 1]. On the contrary, the frequency of stillbirths was statistically highly significantly higher in couples with AA versus SS ($P < 0.001$) genotypes than the normal controls.

The number of neonatal deaths was statistically significantly higher in couples with AS versus sickle cell- β -thalassemia ($P < 0.05$) and highly significantly higher in AS versus SS couples ($P < 0.001$) as compared to controls [Table 1]. However, overall the couples with sickle cell disorders were not significantly ($P > 0.05$, $P < 0.10$) differ from the normal couples with respect to neonatal mortality. Taking into consideration the infant mortality, the couples with sickle cell disorders on the whole and AS versus sickle cell- β -thalassemia showed statistically significantly ($P < 0.05$) higher infant mortality and still higher in AS versus SS couples ($P < 0.001$) than in the controls [Table 1]. However, the carrier couples of sickle cell disease did not show statistically significant ($P < 0.10$, $P > 0.05$) difference from normal controls for infant mortality.

The carrier couples of sickle cell disease, AS versus SS, and on the whole the couples with sickle cell disorders showed statistically highly significant higher mortality under 10 years of age ($P < 0.001$) than the normal controls [Table 1]. There was statistically highly significantly higher number of survival of offspring in couples with AA versus AS, AA versus SS, and sickle cell disorders ($P < 0.001$) than the controls. The carrier couples of sickle cell disease ($P < 0.01$) and AS versus SS couples ($P < 0.05$) also showed significantly higher number of surviving offspring than the controls [Table 1]. There was statistically significantly higher number of live births in couples with AA versus AS ($P < 0.05$) and AA versus SS ($P < 0.001$) than the control couples. However, couples with sickle cell disorders did not show significantly higher number of live births ($P < 0.10$, $P > 0.05$).

The number of abortions per 1000 live births was almost doubled in carrier couples of sickle cell disease (78.4) than in normal controls (44.1) [Table 2]. However, the number of stillbirths per 1000 live births was lower (34.3) in these couples than the controls (40.4).

Neonatal mortality per 1000 live births was almost doubled in carriers of sickle cell disease (34.3) than in controls (14.7).

Table 1: Comparison of reproductive history of carrier couples with and without different sickle cell disorders

Genotypes of couples (diagnosis)	Number of couples (N)	Total number of conceptions	Live births, n (%)	Number of abortions, n (%)	Number of stillbirths, n (%)	Number of neonatal deaths, n (%)	Number of deaths of <1 year, n (%)	Number of deaths <10 years, n (%)	Number 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 (0.0 ^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 ^b)	179 (8.8 ^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 -thal.	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 ^b)	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= $P < 0.01$; c= $P < 0.001$; d= $P < 0.001$, 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 2: Comparison of reproductive history (figures are 1000 live births) of carrier couples with and without different

Genotypes of couples (diagnosis)	Number of couples	Number of conceptions	Number of live births	Number of abortions	Number of stillbirths	Number of neonatal deaths	<1 year deaths	<10 years 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), 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

The neonatal mortality rate was 44 and 33, respectively, for Madhya Pradesh and India for the year 2010 [Table 3].

Infant mortality per 1000 live births was 3-fold higher in carriers of sickle cell disease (44.1) than in controls (14.7). Similar results were obtained in carrier couples of sickle cell disorders mortality for under 10 years of age. The IMR was 62 and 47, respectively, for Madhya Pradesh and India for the year 2011 [Table 3]. The overall neonatal and infant mortality was recorded to be 41.8 and 49.1, respectively, for carrier couples of sickle cell disorders against the controls (14.7 for each category).

While considering the surviving offspring of different sickle cell disorder couples (183), it was observed that the number of normal children (159/367; 43.3%) born to couples with different sickle cell disorders was lower than the defective children (208/367; 56.7%), indicating the progressive increase in defective offspring in these families [Table 4].

DISCUSSION

The findings of the present study are interesting and revealing many practicing aspects of those communities which are genetic victims of recessively inherited hemolytic disorders such as sickle cell disease in Central India. This study strongly supports the contention that hereditary factors in the carrier couples, apart from concomitant non-genetic confounding factors, are responsible for the high fetal wastage in the form of abortions,

Table 3: Comparison of neonatal mortality and infant mortality rates of Madhya Pradesh and India

Genotypes of couples (diagnosis)	Neonatal deaths (neonatal mortality)	<1 year deaths (infant mortality)
AA×AS	36.0	36.0
AA×SS	47.6	47.6
AS×AS	34.3	44.1
AS×SS	214.3	214.3
AS×β-thal. trait	20.4	40.8
AS×S-β-thal.	125.0	125.0
Sickle cell disorders (combined above all)	41.8	49.1
AA×AA (normal)	14.7	14.7
Madhya Pradesh*	44.0 (2010)	62.0 (2011)
India**	33.0 (2010)	47.0 (2011)

Birth to 28 days (neonatal mortality), birth to 365 days or within 1 year (infant mortality), *neonatal mortality rate, Registrar General of India, **infant mortality rate, Sample Registration System, SRS Bulletin December 2011, 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 4: Comparison of surviving children (figures in absolute numbers) of carrier couples with and without different sickle cell disorders

Genotypes of couples (diagnosis)	Couples, n	Conceptions		Live-births		Surviving		Hb AA		Hb AS		Hb SS		S-β-thal.		β-thal. trait	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
AA×AS	65	130	111	105	54	51	39	38	15	13							
AA×SS	18	30	21	20	12	8			12	8							
AS×AS	72	227	204	179	95	84	37	29	14	25	43	31					
AS×SS	9	16	14	11	7	4			3	5	1	2					
AS×β-thal. trait	15	50	49	45	25	20	3	9	6	3			11	2	5	6	
AS×S-β-thal.	4	9	8	7	3	4	0	1	0	1	1	1	1	2	0	0	1
Sickle cell disorders (combined above all)	183	462	407	367	196	171	79	80	52	51	46	32	13	2	5	7	
AA×AA (normal)	200	296	272	264	124	140	124	140									

T: Total, M: Males, F: Females, surviving children with hemoglobinopathies: Normal offspring=159 (43.3%); defectives=208 (56.7%), 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

stillbirths, neonatal and infant mortality, and mortality below 10 years of age in India [Table 1]. These recessively inherited genetic disorders such as sickle cell disease are the outcome of non-viable homozygosity due to inbreeding inadvertently taking place in the vulnerable communities of the region. This is the first 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^[7] in India.

These findings have been supported by the high neonatal mortality rate which was 44 and 33 for Madhya Pradesh and India, respectively, for the year 2010. Neonatal mortality was almost doubled in carriers of sickle cell disease (34.3) than in controls (14.7) per 1000 live births in the present study [Table 2]. Similarly, the IMR of 62 was the highest for Madhya Pradesh as compared to 47 for India for the year 2011. 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 [Table 3].

There are very few studies available on the subject of fetal wastage 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.^[7] 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,^[12] leading to increased homozygosity and reproductive wastage, that is, abortions, stillbirths, neonatal and infant mortality, or childhood mortality.

Looking at the overall scenario of reproductive wastage and survival in different carrier couples of sickle cell disorders, the number of normal children (159/367; 43.3%) born to carrier couples was lower than the defective children (208/367; 56.7%), indicating the progressive increase of defective offspring in these families [Table 4]. This trend shows the lower fitness of the carrier couples or affected families and consequently of the vulnerable population (s).

To bring the reduction/prevention and control of sickle cell disorders in affected families, all the referred and affected families were imparted genetic/marriage counseling to prevent the birth of an abnormal child in their families^[13,14] for the betterment of future generations.

CONCLUSIONS

The increased production of defective (trait and disease) surviving offspring (56.7%) than the normal children (43.3%) leads to increased morbidity and mortality and perhaps may

be contributing toward increased neonatal/infant mortality in Madhya Pradesh. This is the first study that has revealed that hereditary causes, apart from other concomitant non-genetic factors, are also responsible for the high neonatal/infant mortality (reproductive wastage) in the vulnerable population. Further, the progeny of sickle cell disease couples contributes disproportionately to the high neonatal/infant mortality in Madhya Pradesh. Affected families were imparted genetic/marriage counseling. It was envisaged to bring awareness among these couples through genetic/marriage counseling about these 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.

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