PREVALENCE OF THE BETA-S GENE AND SICKLE CELL DISEASE IN INDIA

by

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Introduction: Sickle Cell Disease (SCD), an inherited disorder of the red blood cells, is a major public health problem. India, with a population of 1.2 billion individuals, is estimated to be home to over 50% of the world's patients with SCD. While SCD is common among all ethnic groups in India, high prevalence has been reported in three socio-economically disadvantaged ethnic categories: the Scheduled Castes (SC), the Scheduled Tribes (ST), and Other Backward Class (OBC) groups.

Both the prevalence of the β^s gene and the clinical phenotype of SCD have not been well described in these three population groups.

Objective: Our objective was two-fold: to determine the prevalence of the β^s gene and to describe the clinical phenotype of SCD in the district of Nagpur, Maharashtra located in Central India.

Method: To determine the prevalence of the β^s gene, community screening of target populations was conducted over an eight-year time span. To determine the clinical phenotype of SCD, a cohort of 726 patients was followed over a four and half year time period during which all clinical events presented to the hospital were recorded.

Results

Population Screening: Community wide screening of 35,636 individuals identified 5,437 individuals with sickle cell trait and 1,010 with SCD. The trait prevalence was 13%, 12%, and 3.4% for the SC, ST, and OBC groups respectively.

Clinical Phenotype: A total of 726 patients were followed in the comprehensive clinic over a four and a half year time period. Painful crisis and fever were the most common initial presentations and the leading causes of hospitalizations. These rates were also higher when compared to the published data from the Cooperative Study of Sickle Cell Disease.

Implications for Public Health: The population screening program uncovered previously undiagnosed cases, and provided detailed information for population based disease counseling, prevention programs and comprehensive care programs. Additionally, we present evidence suggesting that SCD may not have milder manifestations in India and underscore the need for detailed studies of phenotype that can form the basis of public health interventions and mechanistic studies of genetic modifiers of clinical phenotype.

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1.0 INTRODUCTION

Sickle cell disease (SCD), the most common single-gene blood disorder (hemoglobinopathy) in the world, represents a significant public health problem in India. In India, SCD is highly prevalent in the Western, Central, and Eastern regions and in pockets of the South in the states of Maharashtra, Madhya Pradesh, Orissa, Andhra Pradesh, Gujarat, Chattisgarh, Tamil Nadu, and Kerala. [1-9] SCD is an inherited disorder of the red blood characterized by vaso-occlusive pain crises, risk for pneumococcal infections, acute chest syndrome, stroke and organ failure and is associated with substantial morbidity and premature mortality. [6]

With a population of over 1.2 billion individuals, it is estimated that India is home to over 50% of the world's SCD patients. [8] While sickle cell is prevalent in many ethnic groups, the highest prevalence appears to be in three particular socio-economically disadvantaged communities, identified as the Scheduled Castes (SC), Scheduled Tribes (ST), and Other Backward Classes (OBC). [8] Each of these categories consists of several different large ethnic groups that have practiced endogamy, or the practice of marrying within a specific group, for millennia and thus represent genetic isolates. In order to be useful for the purposes of planning for genetic counseling services, control programs, clinical service provision or genetic epidemiological studies, screening should generate prevalence estimates of the β^s gene in each ethnic group. The prevalence of the β^s gene has been well described in the ST population;

however, such precise estimates are not available for the OBC population or the SC population. Together these three groups comprise over 75% of the Indian population. [5, 6, 10, 11]

Similarly, the clinical phenotype of SCD in the Indian population has been described mainly in the ST group and not in the SC or OBC groups. Previous studies describe the milder manifestations of SCD in the Indian population compared to that observed in the African and Caribbean populations. [12] Proposed epistatic factors or modifiers contributing to the amelioration of clinical phenotype include the high prevalence of the Arab-Indian β globin haplotype, high Hemoglobin F levels, presence of Xmn1 polymorphic site, and high frequency of alpha Thalassemia in certain population groups. [13-20] However, there has been a lack of detailed phenotypic studies of SCD in various populations in India, and such studies are important not only to understand the clinical and public health impact of the disease in the region but are also essential prior to undertaking mechanistic studies of molecular mechanisms modulating disease severity. [15-17] The Cooperative Study of Sickle Cell Disease conducted in the United States over a 20 year time period is the largest study prospective study of natural history of sickle cell disease conducted to date and has yielded rich data on the clinical course and natural history of disease and has contributed to a variety of mechanistic studies.[13]

Central India, which is one of the high prevalence regions for the β^s gene, has a prevalence rate of 9.4-22.2% for this mutation in various communities. [3, 7] The state of Maharashtra in Central India has a population of 112.3 million of which the SC and ST comprise 10.2% and 8.9% of the population respectively.[11] Additionally, the OBC population comprises 52% of the population.

The purpose of our study was two-fold: to determine the prevalence of the β^s gene and to describe the clinical phenotype SCD in the district of Nagpur, Maharashtra, located in Central India.

2.0 BACKGROUND & SIGNIFICANCE

Sickle cell disease or sickle cell anemia is a hereditary genetic disease that is characterized by the presence of abnormal crescent-shaped red blood cells.[21] While the symptoms related to SCD were known by various names throughout Africa for centuries, it was not until 1910, when James Herrick, a physician, noted the presence of a "peculiar elongated sickle shaped red blood cells" in the blood of an anemic African medical student that the Western world and the scientific community became aware of the disease. [21] A few years after Dr. Herrick's report, Dr. Emmel noted that a patient and her father, both asymptomatic, had sickled blood cells.[22] Furthermore, three of the patient's siblings had died from severe anemia. These observations made by Dr. Emmel were the first to suggest a genetic basis for SCD, although the specific pattern of inheritance was not delineated until the early 1920s. [22] In 1949, Linus Pauling et al. were the first to suggest that the cause of SCD was the result of defective hemoglobin molecules. [23]

Hemoglobin (Hb) molecules are a set of proteins formed by pairing of two alpha (α) and two beta (β) globin polypeptide chains into a tetromeric unit. [24-27] This unit, the $\alpha_2\beta_2$ molecule, forms the major adult hemoglobin responsible for the transport of oxygen from the lungs to tissues. The α-globin gene is located on chromosome 16 and encodes the adult α-globin and the ζ-globin chain, which is the embryonic form of α-globin.[25] The β-globin gene is located on chromosome 11 and encodes four different globin molecules: embryonic ε-globin, fetal γ-globin, adult δ-globin and adult β-globin, all expressed differently during specific times of

development. [25, 27] Figure 1 illustrates the timeline of the expression of the human globin genes from early stages of fetal development to the changes that occur at birth and the first year of life. [25]

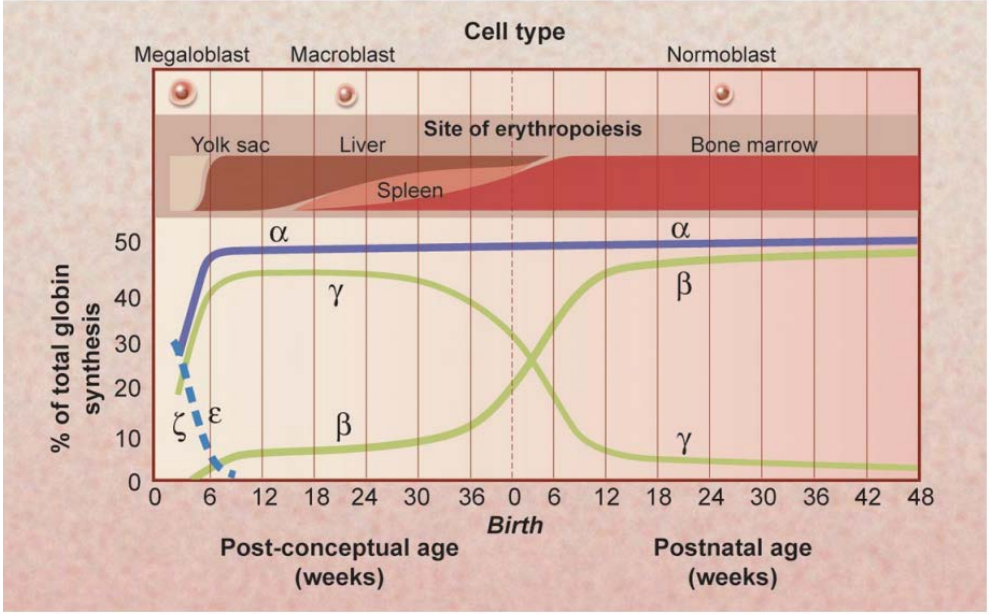

Figure 1. The timeline of human globin gene expression Adapted from Schechter[25]

Hundreds of different structural variants of hemoglobin have been reported in the literature. The most common clinically significant structural variants are HbA, HbA_2, Hb S, HbC, HbF, HbD, and Hb O, shown in Table 1. [26] SCD is used to describe a wide range of hemoglobinopathies that are characterized by Hb S in the presence of another variant beta globin chain. The majority of SCD cases are composed of four primary subtypes: SS, SC, S β^o, and Sβ^+.[26]

Table 1. Different Structural Variants of Hemoglobin

Hb A	$\alpha_2\beta_2$
Hb A_2	$\alpha_2\delta_2$
Hb S	Glutamic acid (GAG) → valine (GTG) at position β^6
Hb C	Glutamic acid (GAG) → lysine (AAG) at

Table 1 continued

	position β^6
Hb F	$\alpha_2\gamma_2$
Hb D Los Angeles or Punjab	Glutamic acid (GAG)→glutamine (CAG) at position β^{121}
Hb O Arab	Glutamic acid (GAG) → lysine (AAG) at position β^{121}
Hb E	Glutamic acid (GAG) → lysine (AAG) at position β^{26}
β^o Thalassemia	Absence of Hb A due to inability to produce normal β-chain
β^+ Thalassemia	Reduced amount of β-chain production leads to variable amount of Hb A.

2.1 SICKLE CELL DISEASE

Sickle cell disease is an autosomal recessive genetic blood disease that affects individuals of African, Mediterranean, Indian, Middle-Eastern, Caribbean, South and Central American ancestry. SCD is characterized by the presence of HbS, caused by a single point mutation involving GAG→ GTG transversion at codon 6 of the β-globin gene.[24, 26-28] This results in an amino acid substitution at position 6 in the β-globin chain from glutamic acid to valine on the surface of the hemoglobin molecule. This amino acid change results in a change in the characteristics of the deoxygenated HS which causes HbS to become less soluble compared to HbA, and when HbS becomes deoxygenated, polymerization of HbS occurs. [24, 26] This polymerization of HbS results in the formation of elongated rigid fibers that damage the cytoskeleton of the red blood cell, causing the shape of the cell to change from the smooth, doughnut-shape to the hallmark sickle-shape. [28-31] (Figure 2) As the red blood cell becomes re-oxygenated the HbS fibers dissociate and revert back to the doughnut-shape. However, as the red blood cell passes through the circulatory system repeatedly, it becomes increasingly more

damaged and the cycles of sickling and unsickling lower the life span of the red blood cell from 120 days to less than 20 days. [24, 26, 29-31]

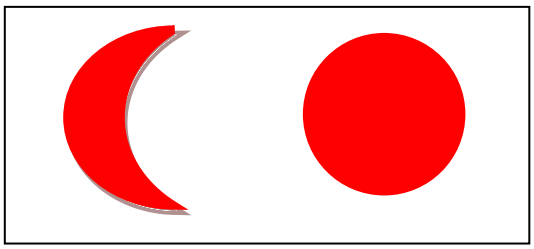

Figure 2. Hallmark Sickle-Shaped red blood cell shape (L) versus normal red blood cell shape(R)

These sickled cells then form homotypic aggregations and heterotypic adhesion to endothelial cells and white blood cells and contribute to the obstruction of blood flow and vaso-occlusion that are hallmark clinical presentations of SCD. [29] This obstruction also results in a lack of adequate blood supply to organs and tissues resulting in damage. The pathophysiology of SCD is illustrated in Figure 3.[23]

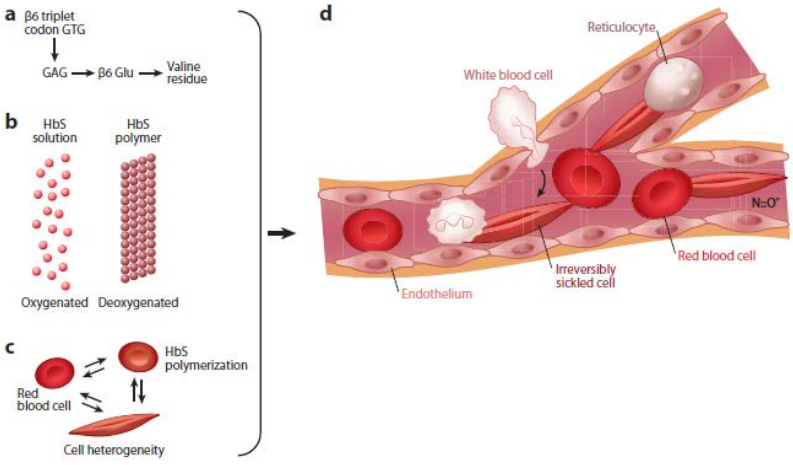

Figure 3. Pathophysiology of SCD

(*a*) Point mutation in sickle cell disease. (*b*) Polymerization of hemoglobin S (HbS) under-deoxygenation. (*c*) Red blood cell shape change in response to HbS polymerization. (*d*) Reticulocyte adhered to endothelium initiates vaso-occlusion by trapping irreversibly sickled cells and forming aggregates with white blood cells.[23]

2.2 INHERITANCE

SCD is inherited in an autosomal recessive pattern, which occurs when an individual inherits one abnormal hemoglobin gene from each parent. If an individual inherits one abnormal hemoglobin gene and one normal gene then he/she is considered to have sickle cell trait or a carrier for SCD. If both parents are carriers for SCD then there is a 25% or 1 in 4 chance in each pregnancy of having a child who will inherit both abnormal hemoglobin genes and present with SCD. [32, 33]

2.3 CLINICAL SYMPTOMS

Clinical symptoms or manifestations of SCD include hemolysis, anemia, pain crisis, infection, acute chest syndrome swelling and vascular occlusion potentially leading to ischemic attacks and organ damage. [34, 35]

2.3.1 Blood

Acute painful crises are the most common type of vaso-occlusive event that occurs in SCD and is the most common reason for admission to hospital for both adults and children. [36] The exact mechanism leading to a pain episode is still unclear although it is thought to be a continuous cycle in which vaso-occlusion results in tissue ischemia and damage, leading to a secondary inflammatory response that triggers the release of norepinephrine which ultimately leads to further tissue ischemia. [37] The severity, frequency, location and duration of pain crises are variable among patients and can be triggered by a number of different events or factors

including: extreme temperatures or changes in humidity, dehydration, stress, alcohol consumption, infection, menses, obstructive sleep apnea and cardiac or pulmonary impairments. [38-47]

The decreased lifespan of the red blood cells (120 days to <20 days) is also responsible for patients to experience chronic anemia with varying degrees of severity. [38-47]

2.3.2 Spleen

An organ to be affected early in SCD is the spleen, which is commonly enlarged during the first decade of life and can undergo progressive atrophy due to repeated attacks of vaso-occlusion and infarction. [48] Individuals with SCD may experience a wide range of splenic involvement from minimal change, splenomegaly, asplenia, and splenic sequestration crisis tor hypersplenism. Splenic complications of SCD are associated with an increased morbidity and in some cases lead to death. [48]

Splenic sequestration crisis results from the rapid sequestration of red blood cells in the spleen; it is a serious complication of SCD and considered to be the second leading cause of death after infection in the first decade of life. [48] Splenic sequestration is characterized by sudden onset of anemia, splenomegaly, evidence of active bone marrow and a decrease in spleen size after blood transfusion. Hypersplenism is defined as splenomegaly with anemia, thrombocytopenia, neutropenia either singly or in combination.[48]

2.3.3 Neurological

SCD is one of the most common causes of cerebrovascular accidents (stroke) in children. While the exact pathophysiology of stroke remains uncertain, contributory factors include anemia, leukocytosis, hypoxemia, abnormal rheology causing endothelial damage, and functional nitric oxide deficiency associated with hemolysis. [27, 28, 36] Approximately 11% of patients will have a stroke by 20 years of age, and once a stroke has occurred, the recurrence risk is greater than 60%. [36, 49]

2.3.4 Pulmonary

Individuals with SCD may present with a variety of chronic manifestations including restrictive and obstructive lung disease, hypoxemia, pulmonary hypertension, airway hyper-reactivity, asthma, or acute chest syndrome. [27, 28, 50-52] Acute chest syndrome is the second most common cause of hospitalizations in SCD and occurs in 40% of patients. [27, 32, 36, 53] Acute chest syndrome is characterized by infiltration of the pulmonary vasculature with sickled red blood cells. Individuals experiencing acute chest syndrome may present with chest pain, tachypnea, fever, rales and rhonchi, leukocytosis, or lobar consolidations. [28, 54]

2.3.5 Infection

A major cause of morbidity and mortality in patients with sickle cell disease is infection. The most common infection causing organism is pneumococcus, and children under the age of three are particularly at an increased risk of infection. [24, 28] Ideally, penicillin prophylaxis is

initiated in the first few months of life as a form of treatment for SCD patients to prevent pneumococcal infection. [24, 28] Impaired splenic functioning, tissue ischemia, micronutrient deficiencies and defects in the activation of the complement pathway are believed to result in an increased risk of bacterial and viral infections. [36, 53] Streptococcus, influenza, salmonella, malaria, hepatitis, parvovirus and chlamydophila are other commonly reported infection in patients with SCD. [27, 28, 36, 53]

2.3.6 Renal/Kidney

One of the main renal complications seen in individuals with sickle cell disease is occlusion of the vasarecta capillaries in the renal medulla, leading to a change in the delivery of oxygen to the kidney, which in turn leads to ischemic damage. [24, 55] Other renal manifestations include hematuria, proteinuria and hypertension, renal infarction, papillary necrosis, renal colic, nephrogenic diabetes insipidus leading to polyuria, focal segmental glomerulosclerosis leading to end-stage renal disease and renal medullary carcinoma.[27, 28, 55]

2.3.7 Ophthalmologic

The blood vessels of the eye may be damaged in patients with SCD due to the physiological changes that occur in the red blood cells. [28, 56] These changes result in the formation of new blood vessels that are weaker and more susceptible to rupture. [28, 56] Depending on the tissue that is involved and the location of the damage, an individual’s vision may or may not be affected. [24, 28] In patients whose vision is affected the primary complications are proliferative retinopathy, retinal artery occlusion, or retinal detachment and hemorrhage. [57, 58]

2.3.8 Growth and Development

The birth weight and length of infants with SCD are typically along the normal growth curve. However, as time progresses, the mean height and weight of individuals with SCD may drop below the 50^{th} percentile, specifically around the age of two. [24, 28] And as the children become older, the growth deficits can become more pronounced. [24, 28] Both females and males may experience delays in sexual maturation and progression through the Tanner stages of development. [24, 28] Females may also experience delays in the onset of menarche while males may experience lower levels of testosterone, dihydrotestosterone, and androsteneion. [24, 28, 47]

2.3.9 Bone

Due to a limited blood supply to some regions of the body, SCD patients may experience bone and joint concerns. Bone complications include bone infarction or osteomyelitis. Osteomyelitis, an acute or chronic bone infection, is most often caused by salmonella. [24, 28] Bone infarctions include dactylitis, commonly known as hand-foot syndrome, which is characterized by painful swelling of the hands and feet that generally resolves within a week. [24, 27, 28, 33, 59, 60] Hand-foot syndrome is typically the first clinical manifestation seen in infants with sickle cell disease between the ages of six months and two years. [24, 28]

3.0 SPECIFIC AIMS

This study has two specific aims:

1) To describe the prevalence of the β^s gene in the scheduled caste, scheduled tribe, and other backward class population in the district of Nagpur, Maharashtra in Central India.
2) To examine the clinical events in a large cohort of patients with SCD, followed in a single center in Nagpur, Maharashtra in Central India to determine the clinical phenotype of SCD in this population.

4.0 METHODS

4.1 POPULATION SCREENING

The Regional Hemoglobinopathy Detection and Management Center (RHDMC) at the Indira Gandhi Medical College located in Nagpur, Maharashtra, has mass community-based and hospital-based screening, management and counseling for sickle cell disease. The institution serves as a referral center with a population of over 10 million individuals in the region and where the high β^s prevalence communities of Scheduled Castes, Scheduled Tribes and Other Backward Classes comprise 55% of the population.

The present study was performed over a span of eight years (2003-2012) and includes the screening of target populations in all age groups (newborns, school age children, young adults, and pregnant mothers) as well as screening in hospital for individuals considered to be at high risk of carrying the β^s gene. Informed consent was obtained from all adults and parents of children once the individuals were educated on the reasoning behind screening. The screening teams were comprised of a clinician, multiple lab technicians and counselors, and volunteers from the villages surrounding the screening camp. Screening camps were conducted in villages wihin a 300km radius around Nagpur that included the areas of Durg, Raipur, Chandrapur, Bhandara, Gondia, Gadhchiroli, Amravati, and Wardha (Figure 4).

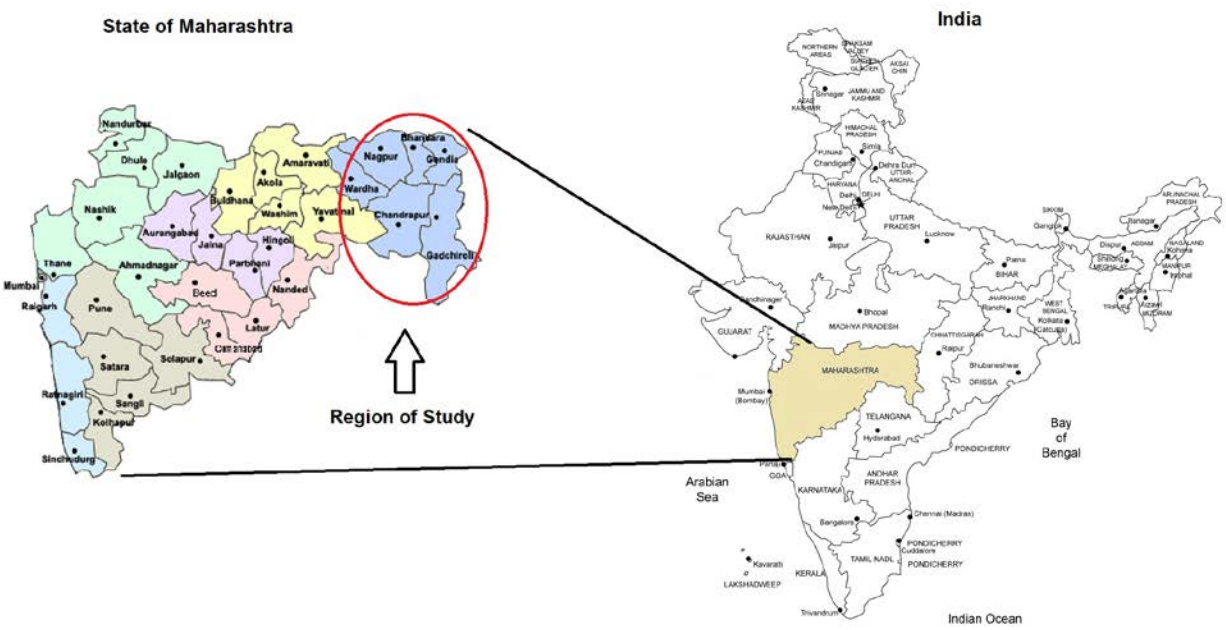

Figure 4. Districts in the State of Maharashtra, India. The circle represents the areas in which community screening camps were conducted. [61]

4.1.1 Screening Procedure

Finger stick samples were taken by the technologists into glass tubes containing reagents (hyper-osmolar phosphate buffer, saponin, reducing agent) for the solubility test, and all positive subjects had 5 ml of venous blood taken into tubes containing EDTA, which were stored in a cooler until return to the base laboratory. Alkaline hemoglobin electrophoresis on agar gel in Tris–EDTA–borate buffer at pH 8.6 was conducted on all solubility positive samples along with known controls for all individuals at the screening camps. [5] High performance liquid chromatography testing was performed on all samples testing positive for HbSS on agar gel electrophoresis and for prenatal screening samples testing for AS or SS. [62] Targeted screening of high risk individuals (family members of individuals identified with sickle cell disease or trait at the screening camps) was completed at the IGMC Hospital in Nagpur using HPLC.

4.1.2 Genotype Results & Counseling

Individuals who were identified through screening to have an abnormal genotype were given identification cards with their name, age, date of birth, village, and genotype (AS/SS). Informational booklets were distributed that contained information about sickle cell disease and trait, inheritance patterns, disease-related issues and premarital counseling. The abnormal genotype cards were distributed by the counselor, who provided genetic counseling and gave additional information and answered questions. Subjects with SS disease were also referred to the onsite clinician, who collected relevant medical history and provided a medical examination. Patients were referred to comprehensive sickle cell clinic for follow up.

4.1.3 Social Classifications

Articles 341 and 342 of the Constitution of India provide statewide lists of communities classified as Scheduled Castes and Scheduled Tribes. Scheduled castes (SC) were historically known as the Untouchables and in the past occupied the lowest status in Indian society. The Scheduled Tribe (ST) populations are aboriginal people known as Adivasis. [63, 64] Other Backward Classes (OBC) are defined in the Indian Constitution as “socially and educationally backward classes” that fall outside the definition of ST and SC. [65] The relative proportion of these social groups in the State of Maharashtra are 10% (SC), 9% (ST) and 19% (OBC).[11]

Within the SC, ST, and OBC there are different ethnic groups that have been identified and classified. The Scheduled Castes and Scheduled Tribes Orders (Amendment) Act, of 1976 classifies the various sub-groups in the SC and ST populations. [65] From this list, the SC and ST populations screened were separated into six and 10 groups respectively, as listed below:

Scheduled Caste-(150 sub-groups within 59 groups)

Group 1: Bhangi, Chambhar, Mehtar
Group 2: Basori, Burad
Group 3: Mang, Matang
Group 4: Bauddha (Mahar)
Group 5: Balai
Group 6: Khatik

Scheduled Tribe(184 sub-groups within 47 groups)

Group 1: Gond, Gowari, Mannewar, Madiya, Mana
Group 2: Kawar, Ratha
Group 3: Adivasi
Group 4: Bhilala, Bhill
Group 5: Halba
Group 6: Kolam
Group 7: Korku
Group 8: Thakur
Group 9: Pardhan
Group 10: Dhangar

The National Commission for Backward Classes Act of 1993 identifies all the different sub-groups within the OBC population in every state of India. [66]

Other Backward Classes(261 groups)

Group 1: Bari
Group 2: Jangum
Group 3: Koli
Group 4: Koshti, Padmashali
Group 5: Kumbhar
Group 6: Kunbi
Group 7: Kasar
Group 8: Navi
Group 9: Dhobi
Group 10: Bhawsar
Group 11: Devang, Sali
Group 12: Shimpi
Group 13: Sonar
Group 14: Sutar
Group 15: Warti
Group 16: Wanjari
Group 17: Sahu, Shahu, Teli
Group 18: Mali, Vanmali
Group 19: Yadav
Group 20: Kochi
Group 21: Gondhali
Group 22: Thakur (Thakkar)
Group 23: Kahar, Kirat
Group 24: Pawar, Powar, Bhoyar
Group 25: Kalar
Group 26: Lingayat
Group 27: Rangari
Group 28: Raut
Group 29: Wani
Group 30: Puri
Group 31: Pahadi

4.2 CLINICAL PHENOTYPE

Subjects for the study were recruited from the pediatric comprehensive sickle cell program at the Government Medical College (GMC) in Nagpur, Maharashtra. Patients have been followed at this center by a single physician (DJ) for over 20 years. They were enrolled in this prospective study of sickle cell phenotype from January 1, 2008, to May 31, 2012. Newborn screening (NBS) was instituted in 2010, and patients detected through NBS were excluded from this analysis. At the time of registration, the diagnosis of SCD was confirmed by standard procedures (cellulose acetate hemoglobin electrophoresis/high performance liquid chromatography) in all subjects. The study was approved by the institutional review board, GMC Nagpur, and written informed consent was obtained from all the patients and/or their parents at enrollment. Individuals lost to follow-up were excluded from analysis. Patients were started on Hydroxyurea (HU) therapy, if they were considered to have a severe phenotype as described in the MSH study. [67] Individuals already on HU therapy at study initiation were excluded, and data were excluded at initiation of HU therapy for patients who started HU during the study and only events before initiation of HU were included. Only events for which the patient was treated in the GMC hospital for medical care were used in the analysis.

4.2.1 Laboratory Diagnosis

The GMC of Nagpur determined the hemoglobin genotype by hemoglobin electrophoresis (Bio-Rad)/ High Performance Liquid Chromatography (Hercules, CA, USA). [62] For those individuals who had received a blood transfusion prior to diagnosis a repeat confirmatory testing

was performed after three months. Complete blood count was performed using an auto-analyzer and reticulocyte count was performed with brilliant cresyl blue staining at the GMC.

4.2.2 Data Collection

These patients were followed up with regularly at the comprehensive sickle cell clinic with all relevant clinical and laboratory data documented in detailed case report forms. Patients were advised to follow up on a monthly basis or as symptoms presented. Patients included in this study received treatment and care, including for acute events, at the GMC, Nagpur. All children age less than five years were offered penicillin prophylaxis. Starting in 2009, the State Government of Maharashtra offered polysaccharide pneumococcal vaccination and *Haemophilus influenzae* type B vaccination free of charge to all patients. Since 2009 all patients between the ages of two and five were offered pneumococcal vaccination. *Haemophilus influenzae* type B, Typhoid, and Hepatitis B vaccines were given as per recommendations of Indian Academy of Pediatrics. [68]

Demographic information about the family was recorded at the initial visit after diagnosis. Clinical records of these patients are available from the time of registration at the sickle cell clinic till the last available follow-up. Data for this study evaluated clinical events from January 1, 2008 to May 30, 2012, or until the last available follow-up within this time frame.

4.2.3 Definition of Events

Only events for which the patient presented to the hospital for medical care were recorded and used for analysis. The event definitions were as follows: a painful event/crisis was defined as pain in the extremities, back, abdomen, chest, or head for which no other explanation could be found. Severe anemia was defined as a hemoglobin level less than 5g/dl. Sequestration crisis was defined as a decrease in the hemoglobin or hematocrit level of at least 20% from baseline accompanied by an increase in palpated spleen size of at least 2 cm from baseline. A febrile episode was defined as a hospitalization with fever (≥ 101º Fahrenheit). Confirmatory malarial diagnosis was defined as an identification of malaria parasite in a peripheral blood smear. Meningitis was defined as abnormal cerebrospinal fluid (CSF) findings and a positive CSF culture. Acute chest syndrome was defined by the following three symptoms: fever, tachypnea, and observation of new pulmonary infiltrates on x-ray. Stroke or a cerebrovascular accident (CVA) was defined as an acute neurologic syndrome secondary to the occlusion of an artery or to hemorrhage with resultant neurologic symptoms and signs.

4.2.4 Statistical Analysis

For assessing demographic information, frequency and percentage were computed for categorical data. For continuous data the median was computed, data categorized into meaningful groups and the frequencies computed within each group. For clinical data each visit with a certain condition was counted to assess its overall frequency. Furthermore the number of patients who had the specific condition at least once during the study period as well as the range

of occurrences of the condition within a person across the study population was compiled. Descriptive statistical analysis was carried out using SAS software 9.3 (Cary, NC, USA).

5.0 RESULTS

5.1 POPULATION SCREENING

Through both community screening and subsequent target screening of high-risk individuals a total of 35,636 individuals was screened, of whom 5,437 were found to have sickle cell trait (SCT) and 1,010 were identified with sickle cell disease (Table 2). A total of 16,832 individuals were screened in the community and targeted high-risk screening of 18,804 individuals was carried out at the IGMC Hospital. Sickle cell trait was identified in 1590 individuals and sickle cell disease was identified in 27 patients through the screening camps. The targeted hospital-based screening of high-risk individuals identified 3,847 individuals with SCT and 983 individuals with SCD.

Table 2. Totals for all groups (Community wide screenings + High Risk Targeted screenings)

	Total	**AA**	**AS**	**SS**
	N	N	N	N
SC-Camps	4399	3824	565	10
SC-Hospital	10380	6930	2744	706
ST- Camps	7430	6559	855	16
ST-Hospital	2624	2047	460	117
OBC-Camps	5003	4832	170	1
OBC-Hospital	5800	4997	643	160
Total	**35636**	29189	**5437**	**1010**

Community wide screening revealed a sickle cell trait (SCT) prevalence of 13% among the SC population, (Table 3), 12% in the ST population (Table 4), and 3.4% in the OBC (Table 5) population.

Within the Scheduled Caste population the sickle cell mutation was most prevalent in the Mahars. In the Scheduled Tribe population, the group consisting of the Gond, Gowari, Mannewar, Madiya, and Mana had the most number of individuals identified. Within the Other Backward Classes group, the Kunbi and Teli groups had the highest number of individuals identified with the β^s gene mutation.

Table 3. Scheduled Caste (SC) Community Wide Screening

	Total	**AA**		**AS**		**SS**	
		N	**(%)**	**N**	**(%)**	**N**	**(%)**
1	172	168	97.7	4	2.33	0	0
2	1	1	100	0	0	0	0
3	78	69	88.5	9	11.54	0	0
4	4058	3507	86.4	541	13.33	10	0.25
5	68	57	83.8	11	16.18	0	0
6	22	22	100	0	0	0	0
Total	4399	3824	86.93	565	***12.84***	10	0.25

1: Bhangi, Chambhar, Mehtar, 2: Basori, Burad, 3: Mang, Matang, 4: Bauddha (Mahar), 5: Balai, 6: Khatik

Sickle Cell Trait Prevalence among the Scheduled Caste Population = 13%

Table 4. Scheduled Tribe (ST) Community Wide Screening

	Total	**AA**		**AS**		**SS**	
		N	**(%)**	**N**	**(%)**	**N**	**(%)**
1	5992	5227	87.23	754	12.58	11	0.18
2	28	26	92.86	2	7.14	0	0
3	71	64	90.14	6	8.45	1	1.41
4	73	62	84.93	11	15.07	0	0
5	216	216	100	0	0	0	0
6	69	64	92.75	5	7.25	0	0

Table 4 continued

7	883	803	90.94	76	8.61	4	0.45
8	2	2	100	0	0	0	0
9	0	0	0	0	0	0	0
10	96	95	98.96	1	1.04	0	0
Total	7430	6559	88.28	855	***11.51***	16	0.22

1: Gond, Gowari, Mannewar, Madiya, Mana, 2: Kawar, Ratha, 3: Adivasi, 4: Bhilala, Bhill, 5: Halba, 6: Kolam, 7: Korku, 8: Thakur, 9: Pardhan, 10: Dhangar

Sickle Cell Trait Prevalence among the Scheduled Tribe Population = 12%

Table 5. Other backward classes (OBC) Community Wide Screening

	Total	**AA**		**AS**		**SS**	
		N	**(%)**	**N**	**(%)**	**N**	**(%)**
1	14	13	92.86	1	7.14	0	0
2	2	2	100	0	0	0	0
3	23	23	100	0	0	0	0
4	96	92	95.83	4	4.17	0	0
5	45	43	95.56	2	4.44	0	0
6	2043	1953	95.59	89	4.36	1	0.05
7	12	12	100	0	0	0	0
8	105	103	98.10	2	1.90	0	0
9	129	126	97.67	3	2.33	0	0
10	14	14	100	0	0	0	0
11	17	17	100	0	0	0	0
12	67	67	100	0	0	0	0
13	174	172	98.85	2	1.15	0	0
14	119	114	95.80	5	4.20	0	0
15	14	14	100	0	0	0	0
16	20	20	100	0	0	0	0
17	1105	1073	97.10	32	2.90	0	0
18	502	486	96.81	16	3.19	0	0
19	60	60	100	0	0	0	0
20	6	6	100	0	0	0	0
21	7	7	100	0	0	0	0
22	100	92	92.00	8	8.00	0	0

Table 5 continued

23	11	10	90.91	1	9.09	0	0
24	156	155	99.36	1	0.64	0	0
25	158	154	97.47	4	2.53	0	0
26	4	4	100	0	0	0	0
Total	5003	4832	96.58	170	***3.40***	1	0.02

1: Bari, 2: Jangum, 3: Koli, 4: Koshti, Padmashali, 5: Kumbhar, 6: Kunbi, 7: Kasar, 8: Navi, 9: Dhobi, 10: Bhawsar, 11: Devang, Sali, 12: Shimpi, 13: Sonar, 14: Sutar, 15: Warti, 16: Wanjari, 17: Sahu, Shahu, Teli, 18: Mali, Vanmali, 19: Yadav, 20: Kochi, 21: Gondhali, 22: Thakur (Thakkar), 23: Kahar, Kirat, 24: Pawar, Powar, Bhoyar, 25: Kalar, 26: Lingayat

Sickle Cell Trait Prevalence among the Other Backward Classes = 3.4%

5.2 CLINICAL PHENOTYPE

A total of 726 patients were followed in the comprehensive clinic; data of 22 were excluded due to death, data of 33 were excluded due to lost to follow up and the data of 25 individuals was excluded at the time they were started on HU. Thirty-nine of these patients were followed for less than six months. Eighty-three percent of the patients have homozygous sickle cell disease (SS), and fifteen percent have Sβ^+ Thalassemia (Sβ^+) or Sβ^0 Thalassemia (Sβ^0). Three hundred and three (42%) patients were female, and 423 patients (58%) were male. Twenty-seven percent of patients were identified under the age of two years, 36% were diagnosed between two and give years of age and 36% were diagnosed after the age of five. There were a total of 1798.4 patient years of observation and 3527 hospital visits recorded.

5.2.1 Population Demographics

The majority of families belong to the lower socio-economic class with a monthly income of over 86% of families being less than a $100. Forty-nine percent of fathers and 55% of mothers

have an education level that is less than or equivalent to a middle school level. Table 6 illustrates the caste distribution within the patient cohort. In this cohort, the majority of patients are part of the SC or "non-tribal" population.

Table 6. Ethnic Group Distribution in the Study Population

Ethnic Group	# of Individuals
Scheduled Caste	481
Scheduled Tribe	72
Other Backward Class	123
Nomadic Tribe	2
Other	30
Unknown	17

5.2.2 Clinical Events

Table 7 illustrates the clinical events observed in the patient cohort. The rates of painful crisis and fever were the most common sickle cell related events in homozygous SCD (SS) and hemoglobin Sβ^+/Sβ^0 patients (overall incidence of 55.88 and 49.27 cases per 100 person-years, respectively). Severe anemia was observed with an incidence of 6.28 cases per 100 person-years and malaria with an incidence of 0.72 cases per 100 person-years. Sequestration crisis and acute chest syndrome were observed with an overall incidence of 1.56 and 0.83 cases per 100 person-years respectively. The incidence rate for stroke and meningitis was 0.28 and 0.11 per 100 person years, respectively.

Table 7. Incidence of Clinical Events per 100 person years

Clinical Event	Nagpur cohort per 100 person years (1771 person years observation)	CSSCD newborn cohort per 100 person years (1851 person years observation)
Painful Crisis	66.52	32.4
Fever	58.32	39.30
Severe Anemia	7.11	4.3
Sequestration Crisis	1.64	3.63
Acute Chest	1.13	24.5
Malaria	0.90	None reported
Death	1.24	1.1

5.2.3 Mortality

Over the four and a half year time period, 22 patients (3.0% or 1.22 cases per 100 person- years) died. The causes of death in this cohort (Table 8) include: infection, splenic sequestration, severe anemia presenting with hypoxic ischemic encephalopathy, and stroke. Thirty-two percent of the recorded deaths are due to unknown causes or deaths that occurred at home for which no causative information is available.

Table 8. Causes of Mortality by Age Distribution

	Infection	Sequestration	Anemia	Stroke	Cardiac Failure
Under 5 years	2	1	1	3	0
5-7 years	0	2	1	0	0
8-10 years	0	0	0	0	0
> 10 years	2	1	1	0	1
Total	4	4	3	3	1

Deaths at Home, Cause Unknown: 7

Total Deaths: 22

6.0 DISCUSSION

6.1 POPULATION SCREENING

Our study for the first time describes the results of community based screening for the prevalence of the β^s gene in different SC groups within Central India. The practice of endogamy in India provides the rationale for the screening of individual populations to better understand the distribution of the β^s gene and guide counseling and awareness programs.

Large scale screening as described in this study has been the foundation for regional disease control programs. [5] While mass screening has provided detailed descriptions for the ST population in previous studies, such detailed descriptions are as yet unavailable for the SC and OBC populations. [1, 3, 4, 6-8, 10, 69-71]

Published studies to date have not distinguished between community-based screening of the general population and secondary screening of family members as a result of detection of a sickle cell mutation in an index case. Secondary screening is likely to reveal a higher prevalence of the β^s gene than in the general population. The multiplicity of ethnic groups and overlapping terminology restricts the utility of prevalence studies. This study addresses these limitations by using standardized terminology as defined by the Census of India [11] and government regulations as well as by grouping ethnic groups that are closely related, have social interactions, and practice inter-marriage to determine the prevalence rates of the β^s gene.

Description of the prevalence of SCT in individual caste groups has immediate practical implications. As seen in the study, using community based screening data to target high-risk individuals has identified a large number of individuals with both sickle cell trait and disease. In lower resource countries, population screening and secondary target screening can identify individuals who may not have otherwise sought care. Identifying these individuals can lead to referrals to comprehensive care clinic to receive the appropriate medicine and follow-up .

The implications for these mass screenings extend beyond identifying carriers of the β^s gene mutation. Emerging data on the potential contribution of sickle cell trait to catastrophic clinical events following extreme physical exertion, such as in military training or professional sporting events, suggest the need for individual awareness of sickle cell trait status. Premarital counseling can be targeted and tailored to the specific groups in which there is a high β^s mutation prevalence. Population-wide screenings can help target specific groups and populations in need of education to raise awareness levels about screening, health implications, treatment, management and pre-marital counseling.

Population screening studies help better define the magnitude of a public health problem. The Mahars, the largest most widespread Scheduled Caste group in India, live in large numbers in an area extending from the Arabian Sea coast in the west to the Bastar plateau in the east, and are a varied group considered to have originated by admixture between the autochthons of the eastern region of Central India and the Proto-Australoid element from the south and constitute around 9% of the total population of Maharashtra. [71, 72] The SC and ST groups with a combined population of 18 million and a trait prevalence of 13% and 12% respectively account for 2.5 million carriers (1.4 million SC & 1.1 million ST) of sickle cell trait in the state of Maharashtra alone. [11] Additionally, the Other Backward Classes comprising 52% of the state's

population account for 1.9 million carries of sickle cell trait in the state. These groups also spill over to neighboring states of Gujarat, Madhya Pradesh and Chattisgarh representing an even larger burden of β^s gene. In comparison, the African American population accounts for 40 million individuals in the United States and with a SCT prevalence of 8%, represent over three million carriers of the β^s gene. [73, 74]

6.2 CLINICAL PHENOTYPE

These findings represent the first report of a large prospectively phenotyped cohort of patients with SCD in India. The clinical picture in the ethnic groups that are present in large numbers in these areas are described and as such must be interpreted as being descriptive of phenotype in these groups only. While various ethnic groups are represented in the patient population, the SC population accounts for a large majority of patients. Vaso-occlusive crisis (VOC) and fever followed by severe anemia are the most common initial presentations and were the leading causes of hospitalizations. The rates of pain crisis and fever in this cohort are higher when compared to the data published from the Cooperative Study of Sickle Cell Disease (CSSCD). [13]

Severe anemia was also more commonly observed than what was reported in CSSCD. [13] The cause of a high rate of severe anemia in patients with SCD in this cohort is unknown. [13] Malaria is a significant co-morbidity in this Indian population. It is possible that malnutrition, infections and malaria may have contributed to the presentation of severe anemia. Further investigation, however, is indicated to better understand this common co-morbidity. It is intriguing that the rate of acute chest syndrome is strikingly lower in this population. This is

particularly surprising in light of the high incidence of pneumonia in the country, with India alone being responsible for over a quarter of the deaths in the world from pneumonia in children under the age of five years. [75, 76] The lower frequency of splenic sequestration is also intriguing. It is possible that this may be reflective of lack of access to care and that splenic sequestration may represent some of the deaths at home with no known cause.

The mortality rate observed in this Indian population (1.24 cases per 100 person-years) is comparable to the observed rate seen in the CSSCD (1.1 cases per 100 person-years). [13] The leading causes of death in this cohort include: infection, splenic sequestration, severe anemia presenting with hypoxic ischemic encephalopathy, stroke and cardiac failure. That nearly one-third of the recorded deaths occurred at home, due to unknown causes may also be reflective of barriers to care for this population.

The high rate of VOC, fever, septic arthritis and unique co-morbidities such as severe anemia and malaria and the high rates of mortality, all suggest that the phenotype of SCD as observed in Nagpur is not milder than that observed in CSSCD. This is in contrast with previous published reports suggesting a milder phenotype of SCD in India. [12] However; these reports were based on cross-sectional studies of a relatively small number of patients. The current analysis underscores the need for careful study of the clinical course of large cohorts of patients prospectively to accurately describe phenotype. That the majority of patients are from low socioeconomic populations with barriers to care is supportive of this possibility.

Environmental and nutritional factors may also contribute to high rate of febrile episodes especially in a low resource country. These along with endemicity of malaria may further contribute to high morbidity and possibly premature mortality in patients with SCD in this region. These findings are also in keeping with our prior retrospective observations. [77] They

are also concordant with cross-sectional observations suggesting high rates of VOC events, infections, organ damage and premature mortality from eastern India. [69] These data provide a compelling rationale for large prospective studies in different ethnic groups in India to determine the contribution of environmental, biological and health systems influences to variation in clinical phenotype.

Median age of diagnosis was four years. This has the potential effect of excluding both those who succumbed to pneumococcal sepsis in early childhood as well as those with mild manifestations who may never present for clinical care at a tertiary care hospital. Further, we excluded patients who were already on HU at the commencement of the study and censored data at initiation of HU on those enrolled in the study in order to describe phenotype modified by HU. Thus, patients with severe phenotype who were started on HU therapy are likely to be under represented in this analysis. These data do, however, indicate that the clinical manifestations of sickle cell disease in India are no less severe than that observed among those who seek care for this disease in the United States.

7.0 CONCLUSION

We provide for the first time detailed prevalence data of the β^s gene among SC and OBC ethnic groups in Maharashtra in Central India. These population screening programs have uncovered previously undiagnosed cases, provided detailed information for population based disease counseling, prevention programs and comprehensive care programs. Over 5,000 individuals were identified with SCT and more than 1,000 individuals were identified with SCD through community-based and target high-risk screening. The population screening study shows how community-based screening can identify individuals who may not have otherwise sought care and guide individuals to available comprehensive care centers. The screening also helped identify specific sub-groups within the SC, ST, and OBC population that have higher prevalence rates of the β^s gene. With a SCT prevalence of 13%, 12% and 3.4% in the SC, ST, and OBC populations respectively, an estimated 4.4 million individuals in the state of Maharashtra alone are expected to have SCT. Similar community-based screening programs need to be initiated throughout regions of India that have a high prevalence of SC, ST, and OBC populations to determine the true prevalence of the β^s gene in India.

Additionally, we present the first evidence suggesting that SCD may not have milder manifestations in India and underscore the need for detailed studies of phenotype that can form the basis of public health interventions as well as mechanistic studies of genetic modifiers of clinical phenotype. Our data shows that the rates of painful crises and fever the two most

common clinical manifestations were observed at higher rates than individuals in the African American population in the United States. Other unique co-morbidities such as severe anemia and malaria were identified that can help direct future research studies to determine exactly how this co-morbidities affect the course of disease. A limitation of this study is that patients were diagnosed when they presented with clinical manifestations of SCD rather on newborn screening. Thus this cohort is not strictly comparable with the CSSCD newborn cohort.

The observation of a severe of phenotype of SCD in India however, has many implications. It makes the case for interventions such as newborn screening and comprehensive care and disease modifying therapy such as HU. The magnitudes of both the at-risk population and the burden of morbidity also suggest the need for public policy to address this major public health problem. This study, by clearly delineating phenotype can serve as the basis for studies of genetic modifiers of phenotype in this population.

The burden of SCD in India has not been well documented or studied thus far. These two studies are of high public health significance and important for the planning of health care delivery services. The study has implications for the need for comprehensive clinical care and further population based prevalence studies in other regions of India to truly understand the impact and burden of SCD in underserved populations.

This thesis reflects the work performed and the author wishes to add a comment.

Comment: Conducting the research for these projects was one of the most unique and greatest learning experiences that I have ever had. I had an opportunity that most students never have the chance to experience and had the honor of meeting and working with four different research groups throughout India on my journey to learn about how Sickle Cell Disease affects individuals in India. I had the chance to attend screening camps and meet individuals from the various villages and every single individual I met was willing to help me learn and teach me all that they knew. Growing up I have made numerous trips to India to visit family members but this project was an eye opening experience and I learned more in my two trips to India for my thesis compared to all the previous trips that I have made before. The sickle cell patients and families

were so friendly, open and willing to talk to me so that I can learn about their experiences. While I hope this work truly provides a framework for future research pertaining to SCD in India it has helped me not only learn about the topic but also about myself and was a phenomenal experience.

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