Brief report on Sickle Cell Disease camps and selected papers on Sickle cell disease research By Dr. Devendra Lingojwar

Sickle Cell Disease (SCD) in an inherited genetic disorder and prevalent in Indian subcontinent including India, Saudi Arabia and Middle East countries and in Western countries in the migrated people of African origin. In USA, people living with homozygous SCD conditions are around 0.1 million whereas in India around 1.4 million, as per DBT data. In India its mainly prevalent in Scheduled Tribes (ST) in most of the states whereas, in few states Scheduled Caste (SC) also suffering with this disease. Its is also present in Other Backward Communities (OBC) in some states.

Dr. Devendra Lingojwar since 1999 to 2014 studied SCD in India, primarily on epidemiology including diagnostic research, prevalence in various communities as well as human parvovirus B19 induced transient aplastic crisis. He studied prevalence in Eastern Maharashtra (Chandrapur and Gadchiroli districts) and Westen Maharastra (Nandurbar district) at different time point and in different states i.e. Kerala (Wynad distict), Chhattishgarh (Baster region Dantewada district and Durg district), Madhya Pradesh (Bhopal). From different field work studies more than 10000 samples were screened from different projects till date. During his post doctoral studies (2014 to 2016) in the Division of Hematology at Albert Einstein College of Medicine, New York he studied research projects involving Development of therapeutic agents for the treatment of SCD in transgenic mice models of SCD.

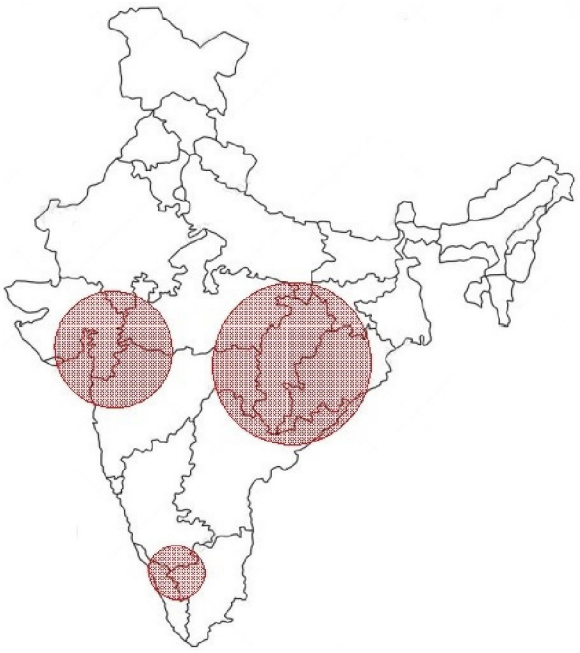
He studied in following institutions for different projects in the field of SCD research as follows:

Research Projects and field studies in Sickle Cell Disease and hemoglobinopathies

1. Intervention program on nutritional and hemoglobinopathies in Indian primitive tribes, Raigad , Western India. (BJ Medical college, Pune and NIIH ICMR, Mumbai India 1999-2000)
2. SCD epidemiology studies in Western and Central India (Maharashtra Aarogya Mandal (MAM) Pune, India (2000-2002) Voluntary contribution in NGO
3. SCD epidemiology studies in Chandrapur city, Central India (MAM Pune, India 2002) Voluntary contribution
4. Prevalence and pathogenesis of human parvovirus B19 in SCD affected tribal population in Central, Western and South India (National Institute of Virology ICMR, Pune, India 2003-2005)
5. SCD prevalence studies in Gadchiroli, Central India (first study, NIV Pune and RESEACH Pune India 2004)
6. SCD prevalence studies in Ballarpur city, Chandrapur district, Central India (first camp in the city, NIV Pune and RESEARCH NGO Pune, India 2004)
7. SCD epidemiology studies of tribal and nontribal population groups from Ballarpur tehsil in Chandrapur district, Central India (first study biggest study conducted as PI at RESEARCH, Pune, India Jul-Dec 2005)
8. Beta thalassemia protein based diagnostic kit development for field studies: Winner of DST Govt. of India: Lockheed Martin Gold medal (Bharati University, Pune, India 2006)

9. Single blood drop technology for diagnosis of sickle cell anemia (ATG LAB, Pune 2008)
10. Variation of abnormal hemoglobins (HbS, Hb E, HbAJ) in Durg, Chattisgarh, Central India (ATG LAB, Pune, India 2013)
11. Development of therapeutic protein based drugs for the treatment of vaso occlusive crisis in SCD in sickle transgenic mice (Albert Einstein College of Medicine, New York, USA 2014-2016)

Field work / lab studies on SCD research in India: From different field work studies more than 10000 samples were screened from different projects till date.



Western zone of India

1. Raigad District: Maharashtra (1999 - 2000) (ICMR multicentric project)
2. Dhadgaon tehsil: Nandurbar district - Maharashtra (2003 to 2004)
3. Haveli tehsil: Pune district – Maharashtra (2006)

Central zone of India

4. Bhopal city: Bhopal district- Madhya Pradesh (1999)
5. Kurkheda tehsil: Gadchiroli district - Maharashtra (2003)
6. Kuwakonda tehsil: Dantewada district: Chhattisgarh (2004)
7. Ballarpur city: Chandrapur district- Maharashtra (2004)
8. Kurkheda tehsil: Gadchiroli district - Maharashtra (2004)
9. Ballarpur tehsil: Chandrapur district- Maharashtra (2005)
10. Chandrapur city: Chandrapur district- Maharashtra (2001)
11. Durg city: Durg district: Chhattisgarh (2013)

Southern zone of India

12. Vythiri tehsil:
    Kalpetta Municipality:
    Wayanad district - Kerala

Training phase

Year 1999: Appointment by ICMR, Training, Lab setup, Field visit and screening in field as well as in NIIH Mumbai and BJ Medical college Pune
Location: Western India, Maharashtra state, Raigad district, Karjat, Village: Neral
This was first exposure of SCD research during Jai Vigyan multicentric five year research project by Indian Council of Medical Research entitled **"Intervention Program for Nutritional Anemia and hemoglobinopathies among some primitive tribal populations of India"**
Funding Agency: ICMR; **Duration: > 1 year** (Sep. 1999 – Oct 2000)
Outcome: This was a multicentric project undertaken at five different places in four states 1.Valsad (Gujarat); 2.Nagpur (Maharashtra); 3.Karjat (Maharashtra); 4.Bhubaneshwar (Orissa) and 5.Nilgiri (Tamil Nadu). ICMR project mployee under BJ Medical College Pune and established field center at Neral, Taluka Karjat. Studied hemoglobinopathies including SCD, thalassemia, and nutritional anemia and G6PD deficiency among primitive tribal population in Western zone of India. Prevalence of nutritional anemia and sickle cell carrier prevalence was established in that region.

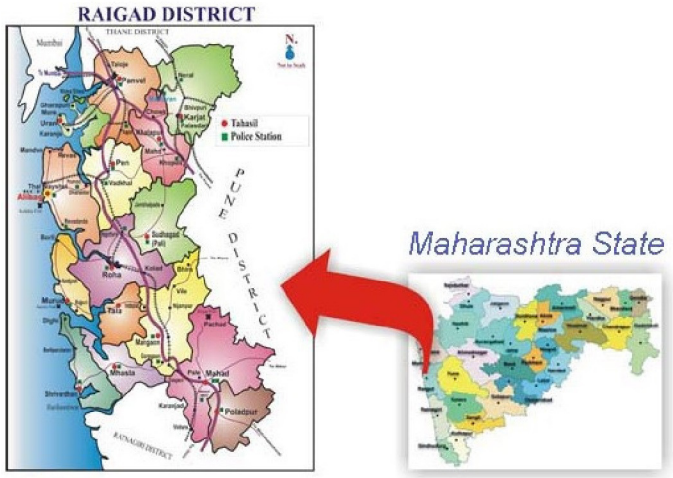


Fig. 1: Study location : Raigad district (Western zone; India)

Duties and contribution: Most of the sickle cell disease prevalence study was based on *Katkari* tribes one of the primitive tribe in Raigad district. Major responsibilities: to set up laboratory in tribal area, school screening program for tribal school children examination for hemoglobinopathies, weekly collection of intravenous blood samples, processing at field station including plasma separation, red cells washing, hemolysate preparation, hemoglobin estimation (Drabkin's method), sickle cell anemia screening by solubility test method, Thassemia testing by NESTROFT method followed by confirmatory test on all samples by Cellulose acetate membrane electrophoresis, Blood grouping, Red cell enzymopathy test: G6PD deficiency test, Parent studies (i.e. Pedigree analysis) of tested patients, report preparation and submission to NIIH ICMR Mumbai. (Sample size studied N=1000). Fetal hemoglobin studies by Singer's method and HbA^2 at headquarter lab at NIIH. Handover of leftover samples for DNA studies to NIIH lab for further studies.

Sickle cell anemia field work project 1:

Year 2001: Field visit

Location: Central India, Maharashtra state, Chandrapur district, Dr. Babasaheb Ambedkar College Chandrapur

Funding agency: BJ Medical College, Pune, MH

Fig. 1: Study location: Chandrapur district (Central zone; India)

Year 2001

Establishment of NGO- Regional Society for Education and Research in Community Health (RESEARCH) Pune,

Year 2003

NGO based workshops and lecturer on sickle cell anemia, collection of old clothes from Pune for donating to SCD patients in Nandurbar district, circulation of sickle cell booklets in local / state language (*Marathi*) for doctors and patients affected by sickle cell gene.

Sickle cell anemia field work project 2:

Year 2003: Field visit (data available)
(Sep, Oct and Dec 2003, and Jan 2004: Total 4 visits)
Location: Western India, Maharashtra state, Nandurbar district, Taluka: Dhadgaon
Funding agency and manpower: NIV ICMR Pune, MAM Pune

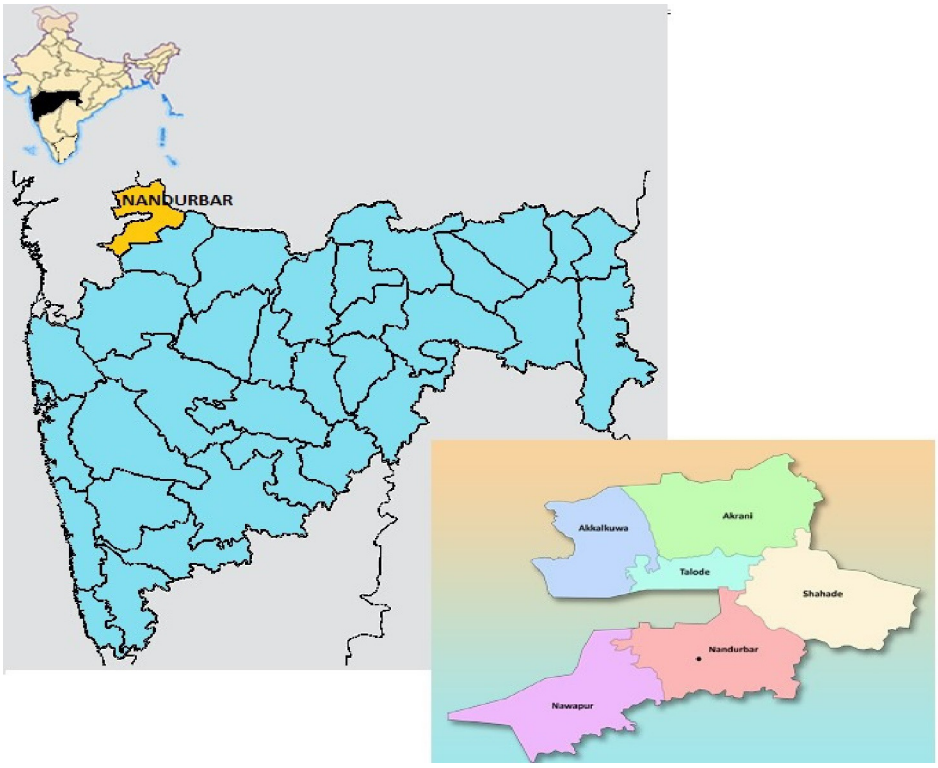


Fig. 3: Study location: Nandurbar district (Western zone; India)

Project outcome:

Total visits in year 2003 (Sep, Oct and Dec.) And 2004 (Feb) = 4
Total samples collected for sickle cell anemia studies = 196
Total samples used only for viral studies = 196
45 Normal individuals (hemoglobin pattern A+A)
25 Sickle cell carriers (hemoglobin pattern A+S)
126 Sickle cell sufferers (SCA/SCD, hemoglobin pattern S+S)
Average Hemoglobin 8.19 gm% in Sickle cell sufferers
All these samples were tested in the field and only separated blood cells and sera transported for virological studies at ICMR Institute.

Human Parvovirus studies in some of the above selected samples: Total tested in recent B19 viral infection studies (IgM) = 112; Total tested in past B19 viral infection studies (IgM) = 127; Total tested in recent B19 viral infection studies (B19 DNA) = 72
Reported first human parvovirus B19 case suffering with **Transient Aplastic Crisis** in Pawra community from Gujarat, (Hb 3.2 gm/dL, B19 IgM, IgG and B19 DNA positive).

Sickle cell anemia field work project 3:

Year 2004: Field visit (data available)

Chattisgarh state

Location: Central India, Chattisgarh state, Bastar region, Dantewada district, kuwakonda block, Halbaras**:** (3rd April 2004 to 14th April 2004)

Funding agency: ICMR**, Participating institutes and human resources**: MAM and RESEARCH Pune, MH, BKNS Halbaras, Chattisgarh

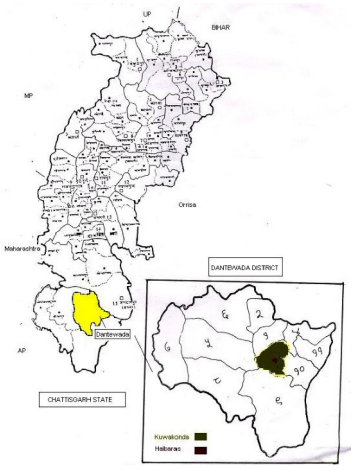


Fig. 4: Field work site, Bastar region, Chattisgarh state

Fig.5: Solubility test: Along with Hemoglobin electrophoresis, this is the golden standard for final laboratory detection of sickle cell anemia in field.

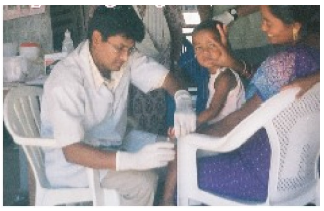


Fig. 6: Pediatric sampling

Fig. 7: With sickle cell anemia affected Families from Chattisgarh

<u>Project out come:</u>

Total blood samples tested = 263; Normal individual (without sickle cell hemoglobin) = 174 (66.16 %); Sickle cell carrier individuals = 82 (31.17 %); Sickle cell sufferer = 7 (2.67 %); Sickle cell anemia carrier prevalence = 31.17 %

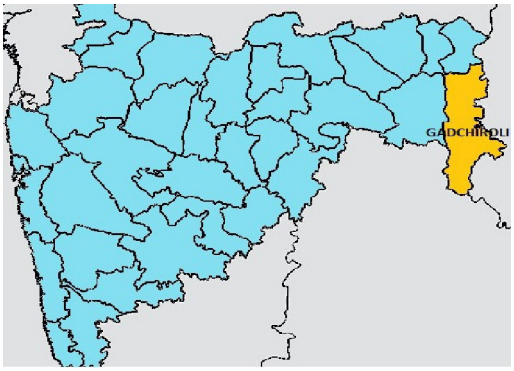
Sickle cell anemia field work project 4:

Maharashtra state: Chandrapur district (data available)
Funding agency ICMR, **manpower and field work facility help:** RESEARCH Pune and Lions Club Ballarpur MH, Chandrapur district: (13th to 15th September 2004) Ballarpur city.

Fig. 8 Study location: Chandrapur district (Central zone; India)

Fig. 9: Counseling in first SCD camp in Ballarpur city

Maharashtra state: Gadchiroli district: : (data available)
Funding agency ICMR, **manpower and field work facility help:** RESEARCH Pune and Aamhi Amchya Aarogyasathi, Kurkheda Dist. Gadchiroli, 20th to 21st September 2004 at Kurkheda



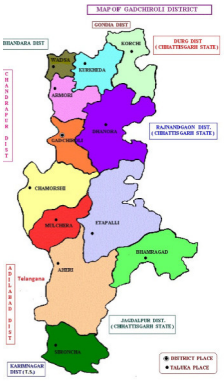


Fig. 10 Study location: Gadchiroli district (Central zone; India)

<u>Project out come:</u>
Total screened 129, Prevalence of sickle cell anemia = 17.82 %, (Chadrapur district)
Total screened 27, Prevalence of sickle cell anemia = 25.92% (Gadchiroli district)

Important note: Four abnormal hemoglobin samples found (other than HbS or HbF as per electrophoresis mobility pattern) but due to lack of facility (Biorad HPLC variant machine and funding limitations) abnormal Hb's could not be reported. Molecular characterization needed that that time to report these non sickle cell abnormal hemoglobin variants.

Sickle cell anemia field work project 5:

2005: Field visit (data available)

Kerala state: Wynad district: 1st January 2005 to 10th January 2005 at Muttil, Kalpetta town, District Wynad.

Population studied: Paniya, Adiya, Kuruma, Chetty (tribals) and OBCs

Funding agency: ICMR, **Human resource and facility for field work**: RESEARCH Pune and Vivekanand Medical Mission, Waynad Kerala

Fig. 11 Study location: Wayanad district (Southern zone; India)

Project outcome:

Total screened and confirmed (solubility test and electrophoresis test) = 102
Normal = 50 (Mean Hb = 9.97); Carriers of sickle cell gene = 15 (Mean Hb = 9.42); Homozygous sickle cell patients (Mean Hb = 7.89) = 37

Summary: Overall hemoglobin level is less than 10.00 in the studied all 3 groups (Normal, carriers and sufferers). Mean hemoglobin for Normal = 9.97 (N=50); Carriers = 9.42 (N=15) and Sufferers = 7.89 (N=37). Low hemoglobin in normal individual may be nutritional anemia. All these samples were tested for viral B19 virus studies and sent to ICMR.

Sickle cell anemia project 6:
Maharashtra state Field visit (data available)

Location: Montfort ITI Ballarpur Dist Chandrapur, Maharashtra state
Title: "Prevalence of sickle cell disease in sixteen villages of Ballarpur Taluka Chandrapur district Maharashtra state"
Funding agency: Catholic Relief Society (CRS), Mumbai
Duration: project duration 1 year
(**Lab studies:** 6 months July to Dec 2005)

Fig. 12: Study Location: Chandrapur district (Central India)

Position: Principle Investigator, **Duties and contributions:** Based on initial 1 week project carried out in Ballarpur city during 2004, detail project submitted for funding to CRS Mumbai. Recruited lab technician, MSW, devoted college students as volunteers, along with ambulance for field work. **Field and laboratory studies**. Medical professional (MBBS, MD) with assistant doctors were also recruited for clinical examination and blood collection. Training were provided to all of them before field work and laboratory studies. We planned 32 visits, (50% villages of only one tehsil covering 10% to 15% random population and one OPD near lab) for rural and tribal SCD investigations.

Fig. 13: Inauguration of Sickle cell camp at Ballarpur by Dr. Devendra Lingojwar Founder President "RESEARCH"

Fig. 14: Sickle cell camp Information by Dr. Devendra Lingojwar During school screening program, Kalmana, Dist Chandrapur

Fig. 15: Sickle cell field work team, this dedicated team including lab technician, MSW, driver and volunteers worked in all 30 field visits

Out of 32 visits, 16 visits were planned for random blood sampling as well as blood sampling of school children. Next 16 visits were planned for follow-up studies. In follow up studies, we collected blood samples from family of either sickle cell sufferers (S+S) or carriers (A+S) for final confirmation by studying pedigree analysis based on parents and sibling's normal beta globin gene (A+A) or sickle cell carrier or

Sufferer (S+S or A+S) gene flow. Reports were given in the form sickle cell card: Total white cards for normal (A+A); half white and half yellow cards for carriers (A+S) and complete yellow cards for sickle cell anemia (SCA) or sickle cell disease (SCD) (i.e. S+S).

Fig.16: Sickle cell field work: School Screening Program

Fig. 17: Sickle cell counseling Dr. Devendra Lingojwar

Fig.18: Sickle cell field work team, with Ambulance, sponsored by MP, Mr. Naresh Pugaliya

Fig.19: Inauguration of Sickle cell field work by MLA, Mr. Sudhir Mungantiwar

Project outcome:

This was the first largest random survey till that time in Chandrapur district at large scale covering 16 locations and 32 field visits including follow-up studies. Out of 5195 registrations, 4008 random population were screened. Total 53 SCD (including two known compound heterozygotes i.e. sickle thal) cases were diagnosed along with 526 sickle cell carriers. Detail proforma of clinical examination and laboratory markers was prepared during and after field work, diagnosis and duing clinical examination by clinicians. Pathogenesis specific clinical history as well as retrospective blood transfusion history was asked. Total registered for project= 5195; Hemoglobin tested for 4939; Note: Individuals with exactly normal hemoglobin and without any clinical features associated with overall anemia were excluded from sickle cell screening test = 256. Solubility test

for screening of presence of hemoglobin S = 4008; Electrophoresis done on 3408 samples. Total carriers = 526; Total sufferers = 53
Sickle cell carrier prevalence in the region = 15.43 %. Prevalence differs caste to caste with category asl also. Overall category wise prevalence was found be SC= 27.43%; ST= 12.6%; OBC=12.23%; NT=2.66%). (Details are mentioned in the conference presentations at the end of this file.)

Sickle cell anemia field work project 7:
Year 4 (2006) – Field visit (data available)
Self funding: By RESEARCH ngo
Maharashtra state (Pune city, PCMC area) -Sickle cell anemia field studies Pune: Sickle cell anemia camp at Pimpale Gurav, Pune, 19th -20th March 2006,

Fig. 20 Study location: Pune district (Eastern zone; India)

<u>Project outcome:</u>
Total screened 202 for anemia studies; Number of samples screened for sickle cell gene 102; Prevalence of sickle cell anemia = 0 %

Sickle cell anemia field work project 8:
Abnormal hemoglobins in (HbS, HbJ and Hb E) in Durg, Chattisgarh

Year 2013: Sickle cell anemia testing was conducted in Durg, Chattisgarh.

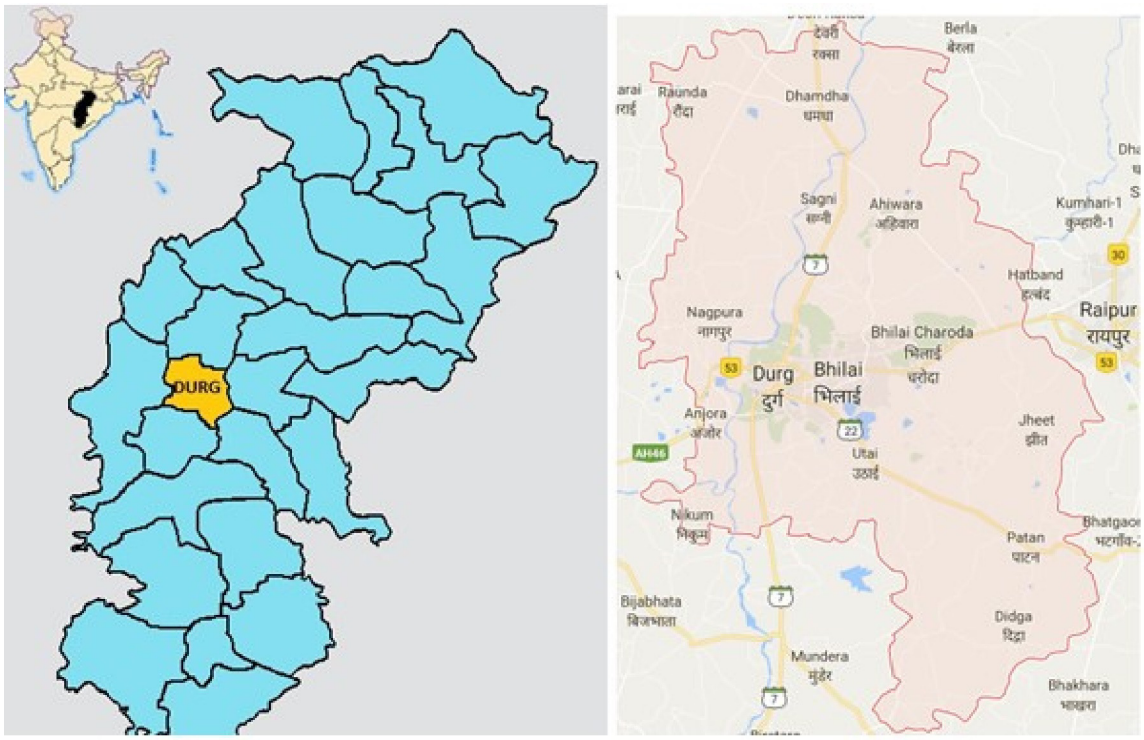


Fig. 21 Study location: Durg district (Central zone; India)

Project outcome:
Among tested samples (N>140), one sample was hemoglobin E. No sickle cell anemia patient was reported in this entire study. However, few carriers were reported.
Among sickle cell carriers, one sample was tested positive for Hb E.

Publication from these projects: Next pages.

Inherent moderate anemia in the community and its possible correlation with frequency of hemolytic events in sickle cell disease: Pilot study from tribal rural Central India

Devendra Lingojwar[1,2,3,*] Ravikant Jadhav[1,2] Prathamesh Kale[1] Lima Hazarika[1] Neeraja Danda[3] Savita Bhutoria[3] Anuja Kapre[1] Sarita Lingojwar[1,2]

1. Molecular Biolology Division, ATG LAB, Biotechnology Research Laboratory, Ganesh Nagar, Pimple Nilakh, Pune 411027
2. Sickle Cell Disease Lab, Regional Society for Education and Research in Community Health (RESEARCH), Ganesh Nagar, Pimple Nilakh, Pune 411027
3. Hematology Division, Department of Medicine, Albert Einstein College of Medicine, Bronx New York USA 10461

Albert Einstein College of Medicine

Introduction

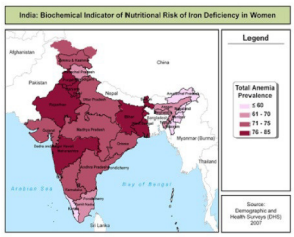


Fig 1: Abundance of Nutritional Anemia in India. (Ref. Demographic and Health Surveys (DHS) 2007)

Indian haplotype is believed to be milder due to alpha thalassemia and fetal hemoglobin (Hb) in tribes. However, severity of illness needs to assess based on socioeconomic, environmental and nutritional status of tribal and non-tribal population groups. Nutritional anemia is highly abundant in India (Fig. 1), It is reported that low iron content in RBC protects normal individuals from the pathogenesis of malaria[1]. It is also briefly reported that access iron loss due to increased urination leads to iron deficiency adults SCD patients and sensitivity of iron detection test by means of serum ferritin is very low in SCD cases and its not easy to correctly identify iron deficiency with this measure.

It is also advised not to supplement iron to SCD female patients if they are pregnant.[2] Kinetics of sickling is highly dependent of MCHC of HbS molecules. Small decrease in MCHC in in SCD cases substantial delay in HbS polymerization. Delay in HbS polymerization helps RBC for fast transit time in the circulation with delay in gelation helps to avoid sickling process.

In this study, theoretical aspects of two important types of pathogenesis were considered for differentiation of SCD phenotypes. In, hemolysis type, the polymerized hemoglobin S attached to each other forming straight rods of 6 to 14 stranded structure, cell gets deformed and most of the free heme is transported to the inner membrane of the red cell. This heme may be toxic to the membrane and that may lead to lysis of cell membrane. Whereas, pain episodes is the final consequence of vaso occlusive crisis. Vaso occlusive crisis is mainly due to clogging of the venules which in turn resulting from inflammation pathways i.e. due to modified membrane of blood cells, which includes cytoadhesion of RBC, WBC and platelets to endothelial cells. In the present study, clinical events of SCD patients were categorized into two phenotypes,[3] i.e. vaso-occlusive Vs hemolytic crisis and analyzed in the background information on Hb levels in the community as per WHO grading system to correlate inherent nutritional anemia in the community with clinical events in SCD patients.

Objective

1. To categorize clinical events of SCD pathogenesis of studied SCD cases into two phenotypes i.e. hemolytic crisis and vaso occlusive crisis
2. To analyze hemoglobin levels in sickle cell carriers and normal population from thee same location as per WHO grading system (No anemia, Mild, Moderate, Severe and Life Threatening anemia)
3. To correlate inherent moderate anemia in AA and AS genotypes with its effect on clinical events of SS genotype for theoretically exploring possible reason of milder form of Indian SCD phenotype

Methods

SCD diagnosis was carried out in 17 different locations (Fig. 2) during July to December 2005 in Ballarpur tehsil, Chandrapur district, Central India, an area known for endemic malaria and were confirmed diagnosed by combination of solubility test (Fig. 3) and cellulose acetate membrane electrophoresis (Fig. 4) at alkaline pH. Some of the known SCD patients, including two compound heterozygotes were also attended the screening program. Hemoglobin estimation was done by Drabkin's method[4]. All the samples were tested by combination of solubility test (which is very specific for HbS)[5,6] and Hemoglobin electrophoresis by cellulose acetate membrane electrophoresis at alkaline pH[7]. Family studies by pedigree analysis from all available data (Fig. 5) were also carried out for final confirmation of homozygous sickle cell patients and heterozygous sickle cell carriers.

International Conference on Revolutionary Laboratory in Modern Biology Diamond Jubilee Celebration, Feb. 15 to 17, 2017 ICMR Mumbai (Poster # P 20)

Fig 2: Location of study: Map of Ballarpur tehsil, Chadrapur district Central India

Clinical events of SCD cases were categorized into hemolytic crisis and vaso occlusive crisis. Cross sectional data of hemoglobin levels of all carriers and normal population were categorized as per WHO grading systems for anemia. Inherent moderate anemia of the community was correlated with clinical events of SCD cases to asses possible contribution of inherent moderate anemia. In this random population study, total males were 1773, average age 14.42 years (1 to 90 years, except one SS patient) and total females were 1635, average age 15.23 years (1 to 80 years). From the confirmed analyzed data (N=3408, AA= 2829, AS= 526 and SCD=63), community-wise and village-wise prevalence was reported based on percent of sickle cell carriers in this region. Prevalence data from this project earlier reported in NIH conference during SCIF 2015 at Washington DC.

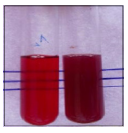


Fig. 3: Solubility test (Representative image)

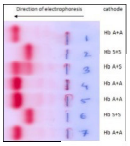


Fig. 4: Hb Electrophoresis (Representative image)

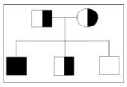


Fig. 5: Family studies (Representative images)

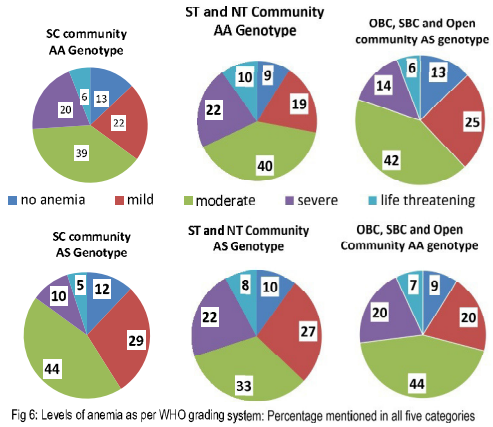


Fig 6: Levels of anemia as per WHO grading system: Percentage mentioned in all five categories

Results

Cross sectional clinical findings suggested that hemolytic events, pallor (in 36 SCD cases) and yellow sclera (31) were found to be more than any single clinical events related to vaso-occlusion based pathogenesis which were noted as joint pain (28), chest pain (18), abdominal pain (16), pain episodes (15), palpable spleen (7), palpable liver (2) and ocular manifestations (9). An assessment by ANOVA with value of $p < 0.05$ was considered statistically significant for the analysis of Hb levels for different age groups as well as genotypes.

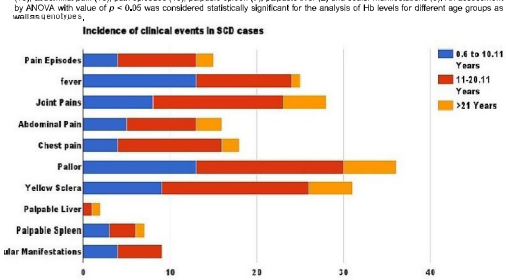


Fig. 7: Clinical spectrum categorized in two phenotypes. Number of events are mentioned on X axis and types of events on Y axis. Comparison of hemolysis Vs vaso occlusive single events suggest that, hemolytic events, Pallor and Yellow Sclera, counted more than any other single vaso occlusive crisis event.

Conclusion

Population survey of hemoglobin levels in normal individuals and sickle cell carriers indicates that moderate anemia is most abundant in this geographic location and in all the studied variables. In homozygous SCD cases, frequency of hemolysis based clinical events, i.e. pallor and yellow sclera were found to be more than that of any other vaso-occlusion based single clinical event, suggesting further need of biochemical analysis of RBCs as well as molecular insight in the studied population.

Acknowledgment

Authors are thankful to: 1. RESEARCH members, students and faculties of ATG LAB. 2. Dr. Tumbade, Br. Mathew and Guru Nanak College of Science, Ballarpur for providing clinical examination, place of lab establishment and volunteers for this project. 3. CRS Mumbai for grant. 4. All voluntary blood donors and participants for this project.

References

1. Adam, I., Anemia, Iron Supplementation and Susceptibility to Plasmodium falciparum Malaria, EBioMedicine (2016).
2. *Kodvin P* Iron in Sickle Cell Disease: A Review Why Less is Better. American Journal of Hematology 73:59 -63 (2003)
3. Kato et al., Deconstructing sickle cell disease: Reappraisal of the role of hemolysis in the development of clinical subphenotypes Blood Rev. 2007 Jan;21(1):37-47
4. *Cook JD. 1985 Measurement of iron status. A report of the International Nutritional Anaemia Consultive Group (INACG). New York: Washington DC, Ch.II; pp 4.*
5. *Cook A and Raper AB, (1971) The solubility test for HbS : a cheap and rapid method. Med Lab Technol 28:4 373-6*
6. *Itano HA, (1953) Solubilities of naturally occurring mixtures of human hemoglobin. Archives of Biochemistry and Biophysics 47:1 148-159*
7. Schneider RG (1973) Developments in the laboratory diagnosis: In sickle cell disease: Diagnosis, management, Education and Research (ed.H.Abramson JF Burties and DL Wethers) 230-43 CV Mosby, St Louis8.

Contact

Dr. Devendra Lingojwar
Director ATG LAB and Founder President "RESEARCH"
Regional Society for Education and Research in Community Health
Saurabha Apartment, Ganesh Nagar, Pimple Nilakh, Pune 411027
+91 20 65104543 +91 9921446321

Sickle cell anaemia carrier prevalence amongst different tribal and non-tribal population groups from Ballarpur, district Chandrapur, Central India

Devendra Lingojwar[1,2,*] Ravikant Jadhav[1,2] Lima Hazarika[1] Prathamesh Kale[1] Chetan Shinde[2] Sarita Lingojwar[1,2]
1. ATG LAB, Biotechnology Research Laboratory, Ganesh Nagar, Pimple Nilakh, Pune 411027
2. Regional Society for Education and Research in Community Health (RESEARCH), Ganesh Nagar, Pimple Nilakh, Pune 411027

Background

Initial studies in Western Maharashtra[1] various groups reported prevalence of sickle cell anemia (SCA) in different parts of the state[2]. Due to tribal status, prevalence studies were explored more in Gadchiroli district. However, neighboring Chandrapur district remained relatively unexplored due to lack of significant sample size, although prevalence in some specific tribal communities and scheduled caste population were reported (Table 1)[2]. Attempts were made to establish SCA prevalence within < 100 sq. km. single geographic region covering >10% of the total population from selected locations from Ballarpur tehsil to establish SCA prevalence at sub-district level in Central India.

Introduction

Malaria and sickle cell disease (SCD) are more common in tribal populations of India, with varying prevalence in different ethnic groups. SCD affects mostly the socioeconomically underprivileged communities, living in the three clusters of 10 neighboring states in India. Most of this endogamous population in India, including, scheduled caste (SC), scheduled tribe (ST), nomadic tribe (NT) and other communities (also known as OBC) are confined to locations, where SCD co-exists with endemic malaria. SCD in tribal population is well known in Central India. Since, these communities are highly endogamous, the study of the prevalence of SCD in all these population groups will be interesting to find out whether there is any trend (increase, decrease or constant) in carrier prevalence as compared to earlier reports from the same geographic region. Due to lack of new born screening facility with molecular testing in Ballarpur tehsil, district Chandrapur (Fig. 1), accurate rate of sickle cell carrier prevalence with its trend is incompletely understood.

Fig 1: Sickle cell anaemia: Three clusters of 10 neighboring states (5-4-3 state model) in India (left); Chandrapur district in Maharashtra state (middle); Tehsil map of Ballarpur (right)

Table 1: Prevalence of SCA in the state of Maharashtra, one of the earlier reported studies (Kate and Lingojwar, 2002).

Communities	Location	Prevalence (%)
Scheduled Caste (SC)	Chandrapur	24
ST (All tribes with SCA in state)	Tribal regions in the state	0-35
Nomadic Tribe (NT)	Nanded/Yewatmal/Osmanabad	5
Other communities (OBCs)	Nagpur/Gadchiroli	4-12

Objective

1. To establish sickle cell anemia prevalence within 100 sq. km. region with 10% the total population screening in the single sub-district region in Central India.
2. To analyze prevalence pattern in tribal and non-tribal communities.

Methods

With the specific aim of analyzing sickle cell carrier prevalence in different communities, 17 geographic locations were selected from Ballarpur tehsil, Chandrapur district, Central India, an area known for endemic malaria and were confirmed diagnosed by combination of solubility test (Fig. 2) and cellulose acetate membrane electrophoresis (Fig. 3) at alkaline pH. Some of the known SCD patients, including two compound heterozygotes were also attended the screening program. Haemoglobin estimation was done by Drabkin's method[3]. All the samples were tested by combination of solubility test (which is very specific for HbS)[4,5] and Haemoglobin electrophoresis by cellulose acetate membrane electrophoresis at alkaline pH[6], which is considered not only the gold standard for resource poor setting in developing countries, where newborn screening is not existing but also one of the two best methods (HPLC and electrophoresis) for new diagnostic kits standardization[7]. Family studies by pedigree analysis from all available data (Fig. 4) were also carried out for final confirmation of homozygous sickle cell patients and heterozygous sickle cell carriers. (Location #17, includes 43% few cases and SCA families from nearby places not included in #1 to #16. Location #16 includes only one area of Ballarpur city i.e. Ravindra Nagar ward 16).*

In this random population study, total males were 1773, average age 14.42 years (1 to 90 years, except one SS patient) and total females were 1635, average age 15.23 years (1 to 80 years). From the confirmed analyzed data (N=3408, AA= 2829, AS= 526 and SCD=53), community-wise and village-wise prevalence was reported based on percent of sickle cell carriers in this region.

Results

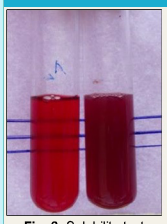


Fig. 2: Solubility test (Representative image)

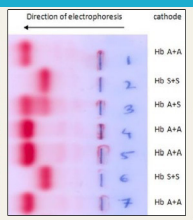


Fig. 3: Hb Electrophoresis (Representative image)

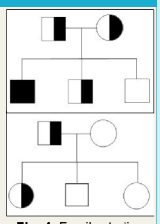


Fig. 4: Family studies (Representative images)

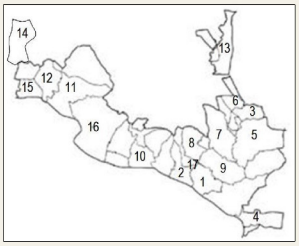


Fig 5: Map of Ballarpur tehsil: 3 urban (#11,12 and 16) and 14 rural locations

Table 2: Village-wise prevalence of SCD based on outcome of this study. Demography and population details were referred from available resources on census[8] (Study duration, July 2005 to December 2005).

Location No. (Village code no.)	Town ward / Village area (sq. km.)	Population density	Population studied (% of total population)	Village-wise Prevalence of SCD (%)
1	6.5	145	115 (12.29 %)	19.13
2	5.3	244	216 (16.67 %)	20.37
3	3.6	686	347 (14.09 %)	16.13
4	6.1	104	79 (12.44 %)	15.18
5	15.3	111	199 (11.74 %)	13.56
6	2.2	470	228 (22.05 %)	9.64
7	10.3	89	85 (9.27 %)	4.70
8	5.6	171	140 (14.5 %)	9.28
9	9.3	259	250 (10.4 %)	16.40
10	4.9	237	123 (10.51 %)	16.26
11	12.5	755	628 (6.68 %)	13.02
12	6.6	581	355 (9.25 %)	14.92
13	7.8	132	40 (3.8 %)	12.50
14	3.6	225	254 (31.71 %)	12.59
15	5.7	142	112 (13.86 %)	11.60
16*	3.0	195	130 (3.60 %)	28.46
17*	3.0	250	107 (14.26%)	35.51*
Total	98.8 sq. km.	297 (mean)	3408 (10.10%)	15.43%

Studied locations mentioned in the table 2 are indicated in the figure 5. Village-wise prevalence (Table 2) and community-wise prevalence (Table 3 and Fig. 6) suggested that, in the tribal population (N=985), sickle cell carrier prevalence is 9.95%. In ST, it is 12.60% and in NT, it is 2.66%. In SC community, (N=853), prevalence of sickle cell carrier is 27.43%. In OBC, (N=1512), sickle cell carrier prevalence is 12.23%. In the non-tribal open category (N=48), carrier prevalence is 8.33%. Overall carrier prevalence of Ballarpur region is 15.43% within less than 100 sq km area screened.

Table 3: Community-wise prevalence of SCA in Ballarpur, Chandrapur, Central India

Population type	Major communities	Total tested Male / Female (M/F)	Heterozygous carriers (M / F)	Homozygous SCD (M / F)	SCA carrier Prevalence (%)
Non Tribe	OBC	1512 (802M / 710F)	185 (92M / 93F)	15 (12M / 3F)	12.23
Non Tribe	SC	853 (415M/ 438F)	234 (110M /124F)	29 (16M / 13F)	27.43
Non Tribe	General	48 (25M / 23F)	4 (0M / 4F)	2 (1M / 1F)	8.33
Non Tribe	SBC	10 (5M / 5F)	5 (3M / 2F)	0 (0M / 0F)	Need more studies
Tribe	ST	722 (394M / 328F)	91 (39M / 52F)	6 (3M / 3F)	12.6
Tribe	NT	263 (132M /131F)	7 (5M / 2F)	1 (1M /0F)	2.66
Total Tribe and Non Tribe	OBC,SBC,SC, ST, NT, General	3408 (1773M / 1635F)	526 (249M / 277F)	53 (33M /20F)	2.66 to 27.43

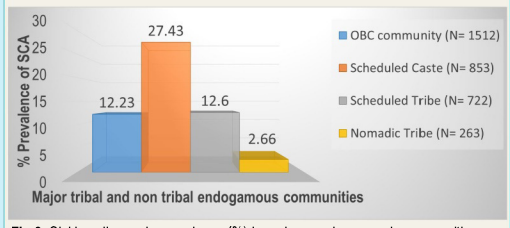


Fig 6: Sickle cell anemia prevalence (%) in various socioeconomic communities.

Conclusion

After comparing with earlier reports, in the state of Maharashtra, these results are found to be consistent for OBC and ST. In SC community, prevalence is increased by >3%. Not much information was available on NT community, as this is the much unexplored group due to their constant migration pattern. However, in our study (N=263), the observed prevalence is 2.66%. As most of the tribal as well as non-tribal population groups are endogamous, i.e. marriage within their community, carrier prevalence percent in this location need to explore with large scale studies based on specific community sampling along with new born screening at birth and compulsory school screening programs before they reach reproductive age for marriage counseling programs.

Acknowledgment

Authors are thankful to: 1. RESEARCH members, students and faculties of ATG LAB. 2. Dr. Jayprakash Tumbade, Prashil Hospital, Br. Mathew, Montfort ITI and Guru Nanak College of Science, Ballarpur for providing clinical examination, place of lab establishment and volunteers for this project. 3. CRS Mumbai for accepting SCD grant proposal. 4. All individual blood sample donors for their participation in this study, without whom this wouldn't be possible.

References

1. Kate SL, Mukherjee BN et al (1978) Red cell glucose 6 phosphate dehydrogenase deficiency and haemoglobin variants among ten endogamous groups of Maharashtra and West Bengal. Hum. Genet. 44, 339-343
2. Kate SL, Lingojwar DP (2002) Epidemiology of sickle cell disorder in the state of Maharashtra. Int. J. Hum. Genet. 2:3 161-167
3. Cook JD. 1985 Measurement of iron status. A report of the International Nutritional Anaemia Consultive Group (INACG). New York: Washington DC, Ch.II: pp 4.
4. Cook A and Raper AB, (1971) The solubility test for HbS : a cheap and rapid method. Med Lab Technol 28:4 373-6
5. Itano HA, (1953) Solubilities of naturally occurring mixtures of human hemoglobin. Archives of Biochemistry and Biophysics 47:1 148-159
6. Schneider RG (1973) Developments in the laboratory diagnosis: In sickle cell disease: Diagnosis, management, Education and Research (ed.H.Abramson JF Burtles and DL Wethers) 230-43 CV Mosby, St Louis
7. Kanter et al. (2015) Validation of a novel point of care testing device for sickle cell disease BMC Medicine 13:2256.
8. Census of India 2001 and 2011. Office of the Registrar General and Census Commissioner. Ministry of Home Affairs, Govt. of India. Available from http://indikosh.com/subd/570326/ballarpur

Contact

Dr. Devendra Lingojwar
Director ATG LAB and Founder President "RESEARCH"
Regional Society for Education and Research in Community Health
Saurabha Apartment, Ganesh Nagar, Pimple Nilakh, Pune 411027
+91 20 65104543 +91 9921446321
www.researchngo.org, www.sicklecellindia.com, www.biotechtrainingproject.com

Lingojwar et al., J Genet Disor Genet Rep 2016, 5:2
http://dx.doi.org/10.4172/2327-5790.1000135

Journal of Genetic Disorders & Genetic Reports

Case Report

A SCITECHNOL JOURNAL

Variation of Abnormal Hemoglobins Concentrated in Durg, Chhattisgarh: A Brief Note Based on Cross-Sectional Study

Devendra Lingojwar[1,2,4*], Pramod Gupta[2], Savita Bhutoria[4], Sarita Lingojwar[1,2], Nikhil Mishra[3] and Anil Kumar[3]

Abstract

Prevalence of different abnormal hemoglobins (Hb) in Indian tribal and nontribal population groups is well established. Sickle cell hemoglobin (HbS) is mostly concentrated in Central and South Indian states, whereas HbD and Hb E is mostly found in North-North-West and North-North-East states respectively. HbJ, an alpha globin gene variant, is earlier reported in North India whereas; its presence in the tribal Chhattisgarh state is not well understood. HbE, a beta-globin gene variant was earlier reported in North Eastern states of India. Prevalence of both these abnormal hemoglobins in the Central India specifically in Durg, Chattisgarh is incompletely understood. In this study attempts were made to analyze the presence of abnormal hemoglobins during screening for sickle cell anemia. Briefly, blood samples (N=44) were analyzed for sickle cell anemia screening and confirmatory tests by solubility tests and cellulose acetate membrane electrophoresis at alkaline pH. Two samples showed an abnormal pattern of separation on cellulose acetate membrane other than HbS. Out of total 44 tested samples, five were sickle cell carriers (HbAS), one was heterozygous HbAJ and the another one was homozygous for HbE while remaining other were normal genotypes i.e. HbAA. In brief, a case of homozygous Hemoglobin E from Kurmi caste of other backward community (OBC) and a different heterozygote pattern, i.e. Hb AJ from Brahmin community is reported from Durg, Chattisgarh, Central India. This study provides possible indication of variation of different abnormal hemoglobins, other than HbS, present in the tribal state of Chhattisgarh.

Keywords

Abnormal hemoglobins; Hemoglobin E; Hemoglobin J; Sickle cell carriers; Indian tribe; Chhattisgarh; Endemic malaria

Case Study

The origin of hemoglobinopathies and prevalence of endemic malaria are inter-linked. Natural protection from malaria pathogenesis after infection might be the most likely and highly accepted hypothesis of the origin of different alleles of normal adult hemoglobin in different population groups throughout the world, more specifically in the tropical countries where malaria is endemic. Mutation in some of the of genes encoding hemoglobin, red cell enzymes and membrane proteins are being extensively studied with reference to protection from *Plasmodium falciparum* [1]. Hemoglobin S is very common in tribal Indian states upto 35% carrier prevalence in states of Maharashtra [2].

HbE is one of the world's most common and important mutation. It results in a heterogeneous group of disorders whose phenotype range from asymptomatic to severe. HbE trait and Hb EE are mild disorders [3]. The Hb E β^{26} (Glu to Lys) is concentrated in parts of South East Asia where malaria is endemic and HbE carrier status has been shown to confer some protection against *P. falciparum* malaria [4]. Pathogenesis study suggested that patients who co-inherit a mild β- thalassemia allele with Hb E may have disease on the mild end of the spectrum while those who co-inherited severe β^{+} or β^{0} thalassaemia alleles might be more severely affected [5]. First reported in Assam with 23% carrier prevalence, HbE is widely distributed in North Eastern states of India with high prevalence amongst 46.4 % in Ahoms of Assam i.e. one of the highest for any abnormal hemoglobin reported from any population in the world. Interestingly, only 1% in Mizoram, 3-33% in West Bengal, while it is almost non-existing in South India [6]. Chhattisgarh is a tribal state and well known for the high prevalence of *P. falciparum* malaria. Presence of abnormal hemoglobin i.e. HbS which is other than normal adult hemoglobin (Hb A), is earlier reported and its prevalence pattern is being studied by different groups [7-10]. Prevalence of this abnormal hemoglobin in Central India specifically in Durg, Chhattisgarh is incompletely understood. Attempts were made to analyze urban samples during sickle cell camp at Durg for finding Hb E in the same population group.

There are more than 50 hemoglobin J variants described in the literature [11]. They all have an electrophoretic mobility "faster" than HbA towards anode in common. All are classified under "variants of the alpha or beta chains" (single or multiple base changes) or "hemoglobins with more than one amino acid substitution in the alpha chain." Hemoglobin J, depending on its type, have different characteristics and functions. Indian variant of Hemoglobin J was reported earlier in North Indian region [12]. Previously Hemoglobin J has been noted by many researchers in various countries. The case of HbJ-Rajappen was reported by Hyde et. al. and later by Henthorn et. Al. in their results of a 10-year program in an English Health region [13,14]. HbJ Baltimore was first described in 1963 in an African-American family. Since then, several cases have been reported in distinct racial groups and also incidentally during the study of other entities, such as thalassemia [15]. More recently, the increasingly frequent determination of HbA1c in diabetic persons has contributed to the appearance of cases of Hb J-Baltimore associated with anomalous HbA1c values [16,17].

In brief, separation of hemoglobin composition was carried out by cellulose acetate membrane electrophoresis method after solubility tests. Briefly, peripheral blood samples (N=44) after red cell washing and solubility testing for screening HbS were processed for hemolysate preparation by osmotic shock method. Pure hemoglobin from hemolysate was subjected to cellulose acetate membrane electrophoresis in Tris-Glycine buffer (pH 8.6). After 45 minutes, resultant hemoglobin pattern was stained by Ponceau S red

***Corresponding author:** Dr. Devendra Lingojwar, Hematology Division, Department of Medicine, Albert Einstein College of Medicine, Bronx NY, USA 10461, Tel: +1 7169512776; E-mail: dplingojwar@gmail.com

Received: April 25, 2016 **Accepted:** May 19, 2016 **Published:** May 19, 2016

dye followed by destaining by 5% acetic acid. Appropriate known controls i.e. sickle cell carrier samples were applied every time new samples loaded on cellulose acetate membrane for electrophoretic separation of samples.

Out of 44 samples as shown in Table 1, five samples showed sickle cell carrier status with one fast migrating (HbA) and one slow migrating band (HbS). Two samples i.e. #17 and #27 (Figures 1 and 2) showed mobility pattern different than HbS. Based on mobility pattern, both samples were compared with normally fast migrating HbA and slow migrating HbS. Homozygous Hemoglobin E formed

Table 1: All three abnormal hemoglobin's along with normal pattern (Hb A+A) shown, around 15% abnormal hemoglobin's (Hb A+S, Hb E+E and Hb A+J) were found in the study.

Gender	Mean age in years	Hemoglobin electrophoresis on cellulose acetate membrane in alkaline conditions			
		HbA+A	HbA+S	HbA+J	HbE+E
Male (n=09)	26.77	09	0	0	0
Female (n=35)	22.08	28	5	1	1
Total (n=44)	23.04	37	5	1	1
Percent prevalence		84.09	11.36	2.27	2.27

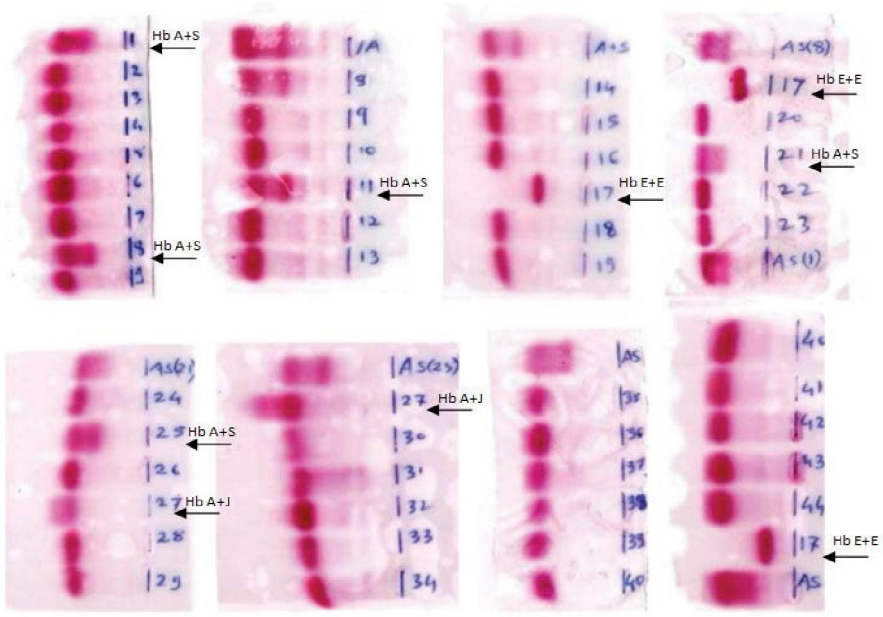


Figure 1: The family tree was shown. The man pointed by an arrow is the proband. The man A had a renal disease but the detail is unclear. The woman B suffers from recurrent self-limiting febrile episodes and arthralgia but the diagnosis is not made. The man C is on hemodialysis. The woman D had a renal transplant.

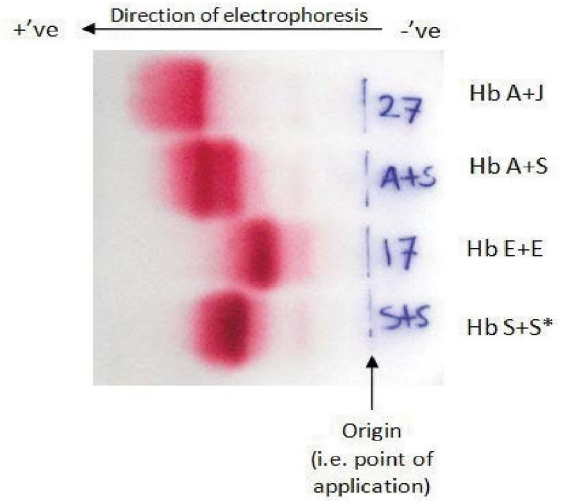


Figure 2: Hemoglobin electrophoresis for sample # 17 and 27. Known A+S sample was applied in between #17 and #27 as appropriate control for referring position of HbA, and HbS on the gel. One homozygous sickle cell disease sample (HbS+S) available in the lab from earlier studies* was also applied below sample #17 as a second positive control for examining position of HbE+E and first positive control HbA+S.

a single band and was very slow during electrophoresis and resolved before HbS whereas, heterozygous HbAJ formed one band with similar mobility pattern like HbA for its majority of fractions and second fast migrating band faster than HbA. Separation pattern of sample no. 17 was different from that of HbA and HbS. After comparison with these abnormal hemoglobins, known mobility of Hb E from review of literature and repeated application during cellulose acetate membrane electrophoresis, it is confirmed that this is Hb E.

In brief, in the present study, a case of homozygous HbE from Kurmi caste of OBC community is found in one 21 year old normal healthy female in Durg Chhattisgarh, Central India. Along with this one abnormal heterozygous case of abnormal hemoglobin variant, HbAJ was also found in one 20 year old healthy female from the Brahmin community without any associated clinical manifestations. As this study was based on very small sample size, the presence of different hemoglobin variants such as HbE and HbJ in single study provides possible indication of variation of different abnormal hemoglobins, other than HbS, present in the tribal state of Chhattisgarh. This initial finding of the presence of abnormal hemoglobin variant in the tribal Chhattisgarh state may prove a significant step for further careful observation of routine sickle cell anemia screening.

Acknowledgement

Authors are thankful to the staff of ATG LAB for their kind help and support for lab studies and Department of Biotechnology, Government V.Y.T. PG. Autonomous College, Durg for their local hospitality and arrangement during the diagnostic field work.

Author contributions

Devendra Lingojwar (DL) contributed to the conception and design of the project, prepared the manuscript and revised it critically for intellectual content. PG, SL and NM has acquired, analyzed and interpreted the data, contributed to data collection and analysis. DL, SB and AK has contributed in image analysis and manuscript preparation. All authors approved the final version of the article.

Grant information

This project was conducted as a part of an intramural project of ATG LAB and no specific grant was used for this project from any funding agency.

References

1. Allison AC (1954) The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria. Trans R Soc Trop Med Hyg 48: 312-318.
2. Kate S, Lingojwar D (2002) Epidemiology of sickle cell disorder in the state of Maharashtra. Int J Hum Genet 2: 161-167.
3. Vichinsky E (2007) Hemoglobin e syndromes. Hematology Am Soc Hematol Educ Program .
4. Ohashi J, Naka I, Patarapotikul J, Hananantachai H, Brittenham G, et al. (2004) Extended linkage disequilibrium surrounding the hemoglobin E variant due to malarial selection. Am J Hum Genet 74: 1198-1208.
5. Winichagoon P, Thonglairoam V, Fucharoen S, Wilairat P, Fukumaki Y, et al. (1993) Severity differences in beta-thalassaemia/haemoglobin E syndromes: implication of genetic factors. Br J Haematol 83: 633-639.
6. Agarwal MB (2005) The burden of haemoglobinopathies in India--time to wake up? J Assoc Physicians India 53: 1017-1018.
7. Panigrahi S, Patra PK, Khodiar PK (2012) Neonatal screening of sickle cell anemia: a preliminary report. Indian J Pediatr 79: 747-750.
8. Balgir RS (2012) Community expansion and gene geography of sickle cell trait and G6PD deficiency, and natural selection against malaria: experience from tribal land of India. Cardiovasc Hematol Agents Med Chem 10: 3-13.
9. Lingojwar D, Kate S, Gore M, Basu A (2004) Prevalence of human parvovirus B19 in some tribal population groups from India. International symposium on Emerging Viral Infections: New Frontiers & Challenges.
10. Bhagat S, Patra PK, Thakur AS (2013) Fetal Haemoglobin and Î²-globin Gene Cluster Haplotypes among Sickle Cell Patients in Chhattisgarh. J Clin Diagn Res 7: 269-272.
11. Patrinos GP, Giardine B, Riemer C, Miller W, Chui DH, et al. (2004) Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res 32: 537-541.
12. Srinivas U, Mahapatra M, Pati HP (2007) Hb J Meerut, a fast-moving hemoglobin: a study of seven cases from India and a review of literature. Am J Hematol 82: 666-667.
13. Hyde RD, Kinderlerer JL, Lehmann H, Hall MD (1971) Haemoglobin J Rajappen; 90 (FG2) Lys leads to Thr. Biochim Biophys Acta 243: 515-519.
14. Almeida AM, Henthorn JS, Davies SC (2001) Neonatal screening for haemoglobinopathies: the results of a 10-year programme in an English Health Region. Br J Haematol 112: 32-35.
15. Arribalzaga K, Ricard MP, Carreño DL, Sanchez J, Gonzalez A, et al. (1996) Hb J-Baltimore [beta 16(A13)Gly-->Asp] associated with beta(+)-thalassemia in a Spanish family. Hemoglobin 20: 79-84.
16. Vandenesch F, Baklouti F, Francina A, Vianey-Liaud C, Bertrand A, et al. (1987) Hemoglobin J-Baltimore (beta 16(A13)Gly----Asp): interference with the assay of HbA1c. Clin Chim Acta 168: 121-128.
17. Barakat O, Krishnan ST, Dhatariya K (2008) Falsely low HbA1c value due to a rare variant of hemoglobin J-Baltimore. Prim Care Diabetes 2: 155-157.

Author Affiliation

Top

[1]*ATG LAB, Biotechnology Research Laboratory, Ganesh Nagar, Pimple Nilakh, Pune, India*

[2]*RESEARCH (Regional Society for Education and Research in Community Health), Pune, India*

[3]*Department of Biotechnology, Govt. V.Y.T. PG. Autonomous College, G.E. Road, Durg Chhattisgarh, India*

[4]*Hematology Division, Department of Medicine, Albert Einstein College of Medicine, Bronx NY, USA*

Int J Hum Genet, 2(3): 161-167 (2002)

Epidemiology of Sickle Cell Disorder in the State of Maharashtra

S. L. Kate and D. P. Lingojwar

Department of Genetics, B.J. Medical College, Pune 411 001, Maharashtra, India

KEY WORDS Sickle cell anaemia; backward communities; Maharashtra.

ABSTRACT Sickle cell disease is a major genetic disorder amongst Scheduled Caste (SC), Scheduled Tribe (ST), and Other Backward Communities (OBC) population groups of Maharashtra. We modified diagnosis technique and developed simple laboratory technology to identify carrier (Hb SS) and sufferer (Hb AS) suitable for field work. In order to find out prevalence for sickle cell disorder we screened major communities from the state and found high prevalence amongst SC, ST and OBC. The overall prevalence amongst SC, ST and OBC is 10%. Severe joint pains and milder type of jaundice are peculiar symptoms amongst sicklers from the state of Maharashtra.

INTRODUCTION

Red blood cells of adult healthy human individual consists of mixture of three unique respiratory proteins known as hemoglobins. One major, with 96% concentration of the total, known as Adult hemoglobin (HbA) and other two minor with less than 2% or traces are Fetal hemoglobin (HbF) and Hemoglobin A_2 (HbA_2). The major function of hemoglobin is to transport oxygen from atmosphere to lungs and finally pass on to all vital organs. The property of combining reversibly with oxygen is unique wonder and interesting. Hemoglobin molecule is conjugated protein and is combination of four hemes and four polypeptide globin chains. Each globin chain is attached to one heme group. There are four different types of globin chains, which are Alpha (α), Beta (β), Gamma (γ) and Delta (δ). Each globin polypeptide chain is a polymer of different amino acids. α globin chain is a polymer of 141 amino acids while β, γ and δ chains each consists of 146 different amino acids. The sequence of amino acids in each globin chain is different and is very specific for that particular globin chain. The pair of α chain is common to all hemoglobins. However in adult hemoglobin (Hb A), the non α chain pair are β globin chains, in fetal hemoglobin (Hb F) pair of γ globin chains and in hemoglobin A_2 (Hb A_2) pair of δ chains.

It can be described as follows:

Hb A = $\alpha_2\beta_2$
Hb F = $\alpha_2\gamma_2$
Hb A_2 = $\alpha_2\delta_2$

The genes for α chains are located on short arm of chromosome number 16 and for β, γ and δ chains genes are located on chrom-osome number 11. The mode of inheritance is autosomal recessive type.

ABNORMAL HEMOGLOBINS

The alteration of sequence of amino acids in either of the four globin chains is termed as abnormal hemoglobin. Abnormal hemoglobins have similar structure of that of normal hemoglobin except slight alteration in the sequence of amino acids and hence may be designated as mutant or variant hemoglobin. A well known example of abnormal hemoglobin is sickle cell hemoglobin (Hb S) in which 6th amino acid (i.e. glutamic acid) is replaced by valine. (Hb S = $\alpha_2\beta_2^{\text{6-Glut-Val}}$). During last fifty years, more than 800 abnormal hemoglobins are reported in the literature. In 90 % of these there is altered sequence of single amino acid in any of the globin chains. Of these, single base mutation reported, 55 % result in β globin chain, 35 % in α globin chain and remaining γ and δ globin chain. In rest of 10 % there may be alteration of two or multiple amino acids or sometimes addition or deletion of amino acids in either of the globin chains. It is observed that β globin gene is most sensitive to single nucleotide base changes. Some of the prominent examples are shown in table number 1.

Abnormal hemoglobins with very high prevalence in world population are Hb C (West Africa); Hb D (North Western India); Hb E (West Bengal and North Eastern India) and Hb S (India, South Africa and Saudi Arabia). Most of the abnormal hemoglobins reported today are not associated with detectable clinical manifestation. Most common clinically relevant

Address for correspondence: Dr. S.L. Kate, Emeritus Medical Scientist (ICMR), 61, Sadhana Society, Hardapsar, Pune 411 028, Maharashtra, India.

Table 1: Prominent examples of abnormal haemoglobin

Replacement of single amino acids	*Replacement of two amino acids*	*Deletion of amino acids*	*Addition of amino acids*
Hb C (β 6th Glut-Lys) Hb D (β 121st Glut-glutamine) Hb E (β 26th Glut-Lys) Hb S(β 6th Glut-Val) Hb Texas (γ 5th Glut-Lys) Hb Indonesia (δ 69th Glut-Arg) Hb Ananthraj (α 11th Lys-Glu) Hb Rampa (α 95th Pro-Ser)	Hb C Harlem (β 6th Glut-Val and β 73rd Asp-Asn)	Hb Gunhill (β chain - amino acid from 92nd to 96th position are deleted)	Hb Tak (β-8 extra amino acids added to C terminal end)

variants are Hb C, Hb D, Hb E, Hb O (Arab) and Hb S (all β chain variants) and occur in polymorphic frequencies in different geographical areas. Amongst all abnormal hemoglobins, Sickle Cell Hemoglobin (Hb S) is more deleterious, since in hypoxic condition it alter the shape of red cells leading to early destruction of the cells and sometime clogging the sickled red cell in microcapillaries producing tremendous, unbearable pain which does not respond to any pain killer. No other abnormal hemoglobin has such ability which ultimately leads to miserable life to patients suffering from sickle cell disease.

Abnormal Hemoglobins Amongst Indian Population

Of the genetic disorders prevalent in this country hemoglobinopathies have been most intensively studied both from case reports and population survey. There are more than dozen abnormal hemoglobins reported amongst different population groups from India. Some are sporadic confined to small community or family. Hb D, Hb E and Hb S are widely spread. Hb D is found amongst Sindhi, Punjabi and Gujrathi population groups with origin in North West India. Hb E amongst Bengali and Assami population groups while Hb S found amongst different population groups from south and central parts of India. Distribution represented in figure 1 (DESH).

Sickle Cell Hemoglobin (Hb S)

In 1904 Prof. Herrick observed that red cells of an African origin anaemic patient acquired sickle like shape instead of normal round shape. After 40 years of research it was found that hemoglobin inside the sickle red cell is mutant variant of normal hemoglobin in which 6th amino acid in β chain is replaced by valine. This was first abnormal hemoglobin reported in literature and labeled as hemoglobin B (Hb B), but because of its sickling property it is relabeled as Sickle Cell Hemoglobin (Hb S). The population survey conducted thereafter found high prevalence of sickle cell hemoglobin in different African tribal groups.

Prior to 1952, no information was available about existence of Sickle Cell Hemoglobin in India. In 1952 it was recorded for the first time simultaneously amongst tribal population groups of Nilgiri Hills and laborers in the tea gardens of Assam[1,2]. Now it is firmly established that this gene harbor amongst different caste groups but very high prevalence amongst Scheduled Caste (SC), Scheduled Tribe (ST) and Other Backward Communities (OBC).[3-15]

Sickle Cell Disorder Scenario in the State of Maharashtra

Taking into our huge population size, more than 50 % of the world's sickle cell anaemia cases are in India. It is estimated that most of the cases are in the Central and South India. During last 50 years, because of simple, reliable and inexpensive laboratory methods are available[18], the large number of population genetic surveys conducted by different scientific groups and data on geographical distribution, clinical manifestation along with its variations, available from the state of Maharashtra [3-15].

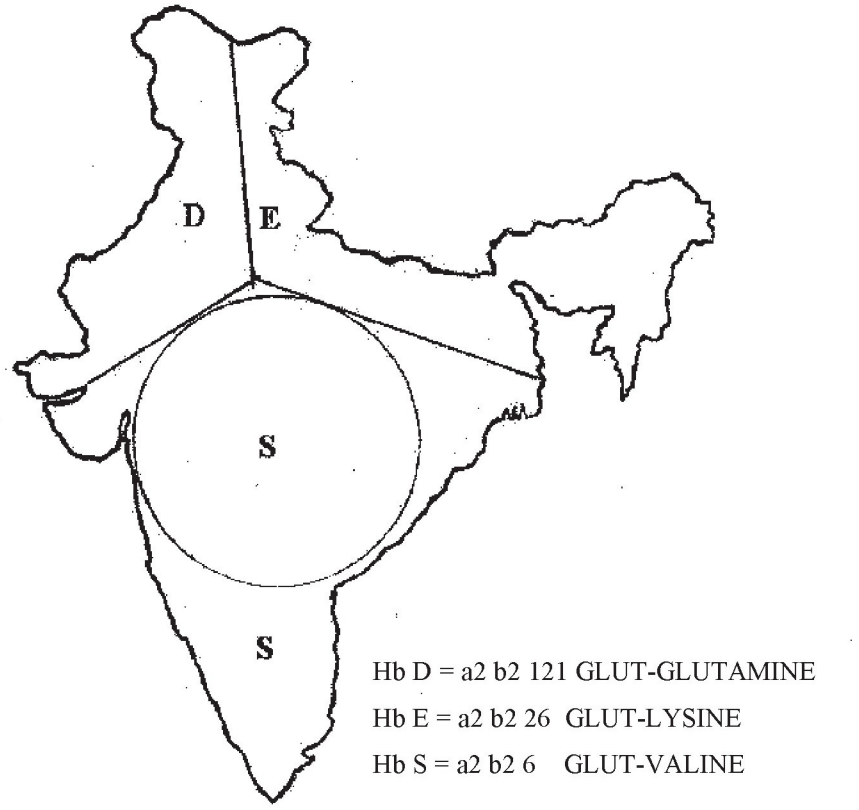


Fig. 1. Distribution of abnormal haemoglobin in India–DESH (D, E and S Haemoglobins)

METHODOLOGY

1. Solubility Test: Deoxygenated Sickle cell hemoglobin has an abnormally low solubility. A fibrous precipitate is formed when a concentrated solution of sickle cell hemoglobin is deoxygenated (This precipitate deforms red cells and gives them their sickle shape. The rate of fiber formation is proportional to about the tenth power of the effective concentration of deoxyhemoglobin S. Thus, fiber formation is a highly concerted reaction)[19]. HbS is deoxygenated form and is insoluble in phosphate buffer (giving turbidity to the solution) while other hemoglobins are completely soluble (giving clear solution).

2. Electrophoresis of Hemoglobin: Each of the major hemoglobin types has an electrical charge of a different degree, so the most useful method for separating and measuring normal and abnormal hemoglobins is electrophoresis. This process involves subjecting hemoglobin components from dissolved red blood cells to an electrical field. The components then move away from each other at different rates, and when separated, form a series of distinctly pigmented bands. The bands are then compared with the other samples on the same membrane strip called as control. Quantitation of different hemoglobins can also be made to indicate severity of any abnormality.

Electrophoresis of Hemoglobin at Alkaline pH (pH 8.6) Using Cellulose Acetate Membrane as Supporting Medium: Hb A has faster mobility than Hb A which is slower. Hb D and Hb S have similar mobility in between Hb A and Hb A_2. In case of Hb S solubility test is positive.

Criteria used is as follows:

Combination of electrophoretic technique with solubility test is a golden standard for detecting sickle cell hemoglobin in carrier and sufferer state. It is very cost effective (about

Rs.10/- per hemoglobin blood samples) hence screening on large scale can be undertaken by different institutions[18].

From the available data [5-10, 14.] it is found that Sickle cell gene is widely spread in all districts of Eastern Maharashtra (known as Vidarbha region), North Maharashtra (Satpuda ranges) and some parts of Marathwada region [16, 17.]

Table 2: Methods used for identification of haemoglobins

Solubility test	*Electrophoretic mobility (major bands)*	*Genotype*
+ ve	A+S	Heterozygote (Carrier)
+ ve	S+S (One single band at S position)	Homozygote (Sufferer)
-ve	A+A (One single band at A position)	Normal

Table 3: Prevalence for sickle cell disorder (carrier) amongst Scheduled Tribe population groups (State of Maharashtra).

Tribe	*District*	*Prevalence %*
Otkar	Gadchiroli	35
Pardhan	Gadchiroli, Chandrapur, Yewatmal	32
Pawara	Nandurbar, Jalgaon	25
Madia	Gadchirli	20
Bhill	Nandurbar	20
Halbi	Gadchiroli	13
Rajgond	Gadchiroli	11
Korku	Amravati	10
Kolam	Yewatmal	09
Warli	Thane	09
Katkari	Pune, Raigad	07
Kokana	Nashik	04
Andha	Nanded	02
Mahadeo Koli	Pune, Nashik	01
Thakar	Pune, Raigad	01
Paradhi	Solapur	00

EPIDEMIOLOGICAL SUMMARY OF SICKLE CELL DISORDER IN THE STATE OF MAHARASHTRA

Sickle Cell Anemia is a single point mutation red cell hereditary disorder. It is an autosomal recessive disorder and hence occur in two forms i.e. Carriers (AS type) and Sufferers (SS type). Haploype studies suggest that in majority of cases it is Arab-Indian haplotype. This disorder is mostly confined to economically and socially backward commu-

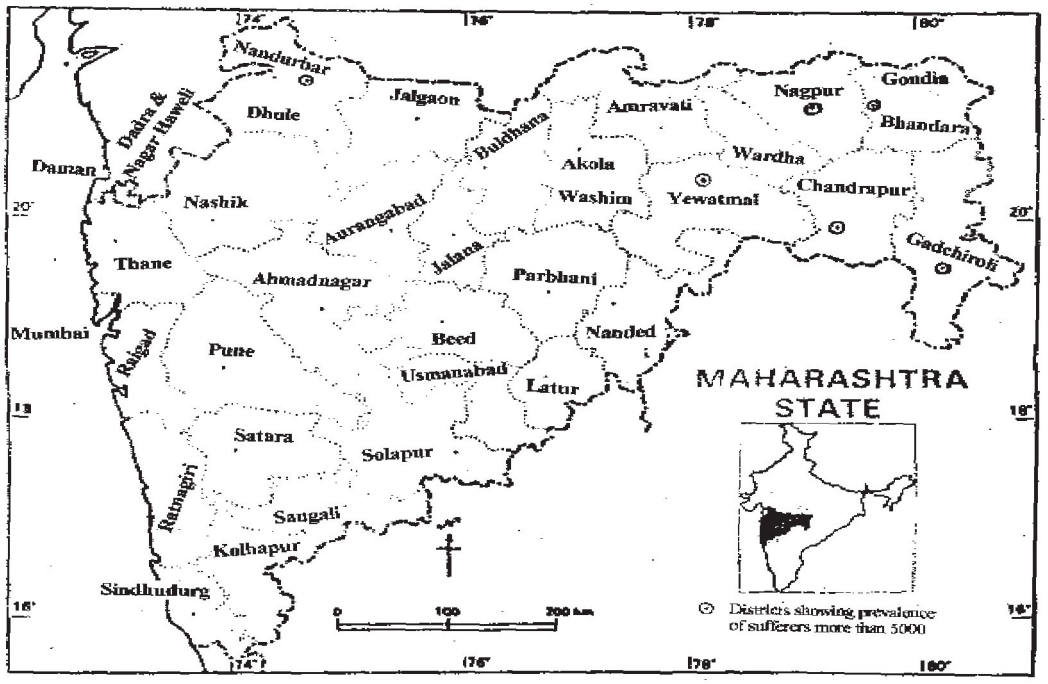


Fig. 2. Maharashtra state:⊙ Districts showing prevalence of sufferers more than 5000

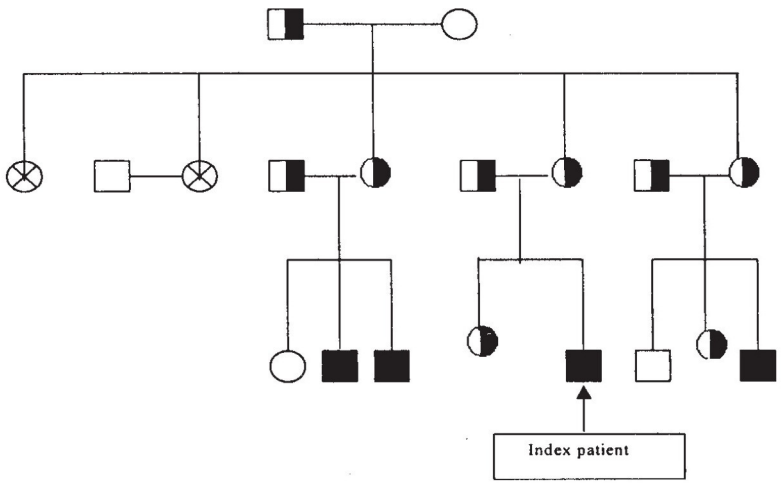


Fig. 3a. Typical pedigree from Vidarbha region

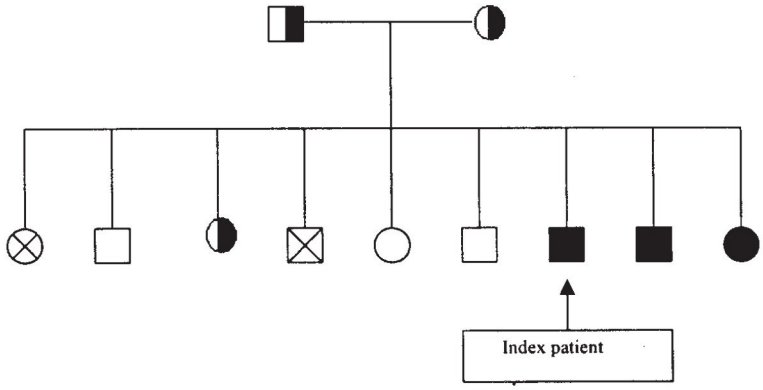


Fig. 3b. Typical pedigree from Nandurbar district

Table 4: Prevalence of sickle cell disorder (carrier) amongst scheduled caste population (State of Maharashtra)

District	*Prevalence*
Chandrapur	24
Gadchiroli	23
Nagpur	22
Bhandara	18
Gondia	15
Thane	12
Wardha	07
Washim	07
Aurangabad	07
Nandurbar	05
Pune	04

nities known as Scheduled Caste (SC), Scheduled Tribe (ST) and Other Backward Communities (OBC) groups. It is rare in other communities.

Table 5: Prevalence of sickle cell disorder (carrier) amongst OBC population groups (State of Maharashtra)

Groups	*District*	*Prevalence%*
Teli	Gadchiroli	12
Teli	Nagpur	10
Kunbi	Gadchiroli	10
Kunbi	Nagpur	04
Banjara	Nanded	05
	Yewantmal	
	Osmanabad	

Expected Carriers and Sufferers in the State are as Follows

Total population of Maharashtra (Census 2001)	100 millions
Total S.C., S.T., and O.B.C. population	25 millions

Expected carriers (10%) 2.5 millions
Expected sufferer (0.5 %) 0.125 millions
(Rough estimates as per available record from 2001 census).

It is also estimated that the districts with more than 5000 cases of sickle cell anaemia are Gadchiroli, Chandrapur, Nagpur, Bhandara, Yewatmal and Nandurbar districts. (Fig. 2).

The common clinical features observed among sickle cell anaemia cases are Anaemia (Moderate type), Intermittent jaundice (yellow sclera) figure 4, Joint pains (severe),Vaso-occlusive crisis (painful) and Spleenomegaly. Intermittent jaundice and joint pains are characteristic of the disease. These symptoms usually visible at the age of 3 to 4 years and

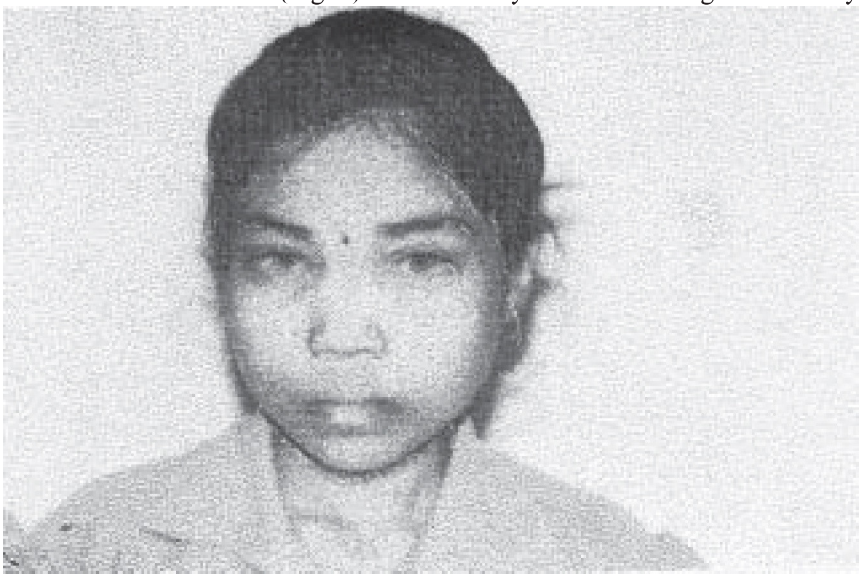


Fig. 4. Sickle cell anaemia patient (HbSS) showing intermittent jaundice (yellow sclera)

Fig. 5. A family with three sickle cell Diseased patients (Hb SS) from hill tribal community, Dhadgaon

severity increases with the age. Extreme hot and cold and unexpected changes in the atmosphere aggravate the symptoms. Though rare, most of the complication recorded in the world literature are also found in few cases. Cholelithosis and Hand and foot syndrome observed in few cases. There is a large variation in clinical presentation. Some are mild some are severe type. Clinically severe cases are found in Eastern Maharashtra than other parts of the state.

Avascular necrosis of bones and grade I early proliferative changes involving periphery of the retina are observed in elderly patients. Foot ulcers and priapism not observed. Pregnancy lost i.e. repeated abortion in family where both parents are carriers are recorded. Carriers are usually asymptomatic except few cases of painless hematuria. High prevalence observed in malaria endemic areas.

Compound heterozygotes are seen frequently i.e. S-β thalassaemia. It is common amongst non-tribal and non-scheduled caste groups.

The disease is incurable and hence patients are not only physically affected but mentally too. Due to presence of sickle cell anaemic patient whole family is disturbed. (Fig.3 and 5).

High prevalence is observed in the rural area from Eastern part of Maharashtra and hence population is at high risk in this area. In this rural area general practitioners have very little knowledge about this disease. Moreover, diagnostic and treatment facilities are not available. Modern interventions like Bone Marrow Transplantation (BMT), Gene Therapy (GT), Preimplantation Genetic Diagnosis (PGD) and Prenatal diagnosis is beyond their capacity (the population with sickle cell anaemia inheritance). Lack of knowledge and awareness enhances superstitions about the disease.

It is necessary to establish community control programme involving people, doctors, social workers, and sympathizers. This programme will undertake diagnosis, treatment, management and counselling. Government of Maharashtra is aware of these facts but unable to undertake major projects because of financial constraint. Similarly there is need to have Central Institute to study epidemiology and clinical course aspects in detail. It needs support from Central agencies.

REFERENCES

1. Balgir RS 1996. The prevalence of sickle cell haemog-lobinopathy in India. In: *The Encyclopedia of Dravidian Tribes*. Vol. 1 T. Madhava Menon, C Siva Thanu, K.P.Prasanth, M. Sasikumar and PRG Mathur (Eds.). Trivandrum: The International School of Dravidian Linguistics pp 21-29.
2. Banker MP, Kate SL, Mokashi GD, Khedkar VA, Phadke MA 1984. Distribution of sickle cell haemoglobin among different tribal population groups in Maharashtra. *Indian J Haematology*, **11**: 4.
3. Bhatia HM, RaoVR 1987. *Genetic Atlas of Indian Tribes*. Bombay: Indian Council of Medical Research.
4. Deshmukh VV 1963. Deficiency of erythrocyte glucose 6 phosphate dehydrogenase and sickle cell trait a survey at Aurangabad, Maharashtra. *Ind J Med Res,* **56**: 821.
5. Dunlop KJ, Mazumdar UK 1952. The occurrence of sickle cell anemia among a group of tea garden laboures in upper Assam. *Indian Medical Gazette*, **87**: 387-391.
6. Kate SL 2001. Health problems of tribal population groups from state of Maharashtra. *Ind J Med Sci,* **5(2):** 99-108.
7. Kate SL, Khedkar VA, Mukherjee BN 1976. Cellulose acetate membrane electrophoresis- Simple rapid inexpensive method for detection of haemoglobin variants. *Indian J Phy Anthrop Hum Genet,* **2**: 123.
8. Kate SL, Mukherjee BN, Malhotra KC, Phadke MA, Sainani GS, Mutalik GS— Red cell Glucose 6 phosphate dehydrogenase deficiency and haemoglobin variants among some caste and tribes from Maharashtra and West Bengal. *Annual conference of Indian Soc. of Hum. Genet. Calcutta.*
9. Lehmann H, Vatbush M 1952. Sickle Cell Trait in Southern India. *British Medical Journal*, **1**: 289-290.
10. Lele RD, Solanki BR, Bhagwat RB, Ingle VN, Shah PM 1962. Haemoglobinpthies in Aurangabad region. *J Assoc Physicians India*, **10**: 263.
11. Mohanty D, Pathare AV 1998. Sickle cell anemia-The Indian scenario. *Ind J Hematol,* **16(1):** 1-2.
12. Mukherjee BN, Malhotra KC, Das SK, Mazumdar PP ,Roy M, Kate SL, Sainani GS 1979. Genetic polym-orphism analysis amongst endogamous population of Maharashtra – India. *J Human Evolution*, **8**: 555.
13. Negi RS 1968. Sickle cell trait in India. A review of known distribution. *Bulletin Anthrop Survey of India*, **17**: 439.
14. Rao VR, Gorakshkar AC 1990. Sickle cell haemoglobin, beta thalassemia and G6PD deficiencyin tribes of Maharashtra – India. *Gene Geography,* **4**: 131.
15. Sharma A 1983. Haemoglobinopathies in India. Peoples of India. *XV International Congress of Genetics,* New Delhi India Dec. 12-21.
16. Sharma RS, Parekh JG, Shah KM 1963. Haemoglobinopathies in Western India. *J Assoc Physician India,* **11**: 969.
17. Shukla RN, Solanki BR 1958. Sickle cell trait in central India. *Lancet,* 297.
18. Stryer L 1995. *Biochemistry*, IV ed., New York: W.H. Freeman Co. 171-172.
19. Sukumaran PK 1974. Abnormal hemoglobins in India. In: *Trends in Hematology.* NM Sen (Ed.) Calcutta: J.B. Chatterjee Memorial committee, pp. 225-261.