Brief report of Sickle Cell Diseaes studies by Dr. Devendra Lingojwar (1999-2017)

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.

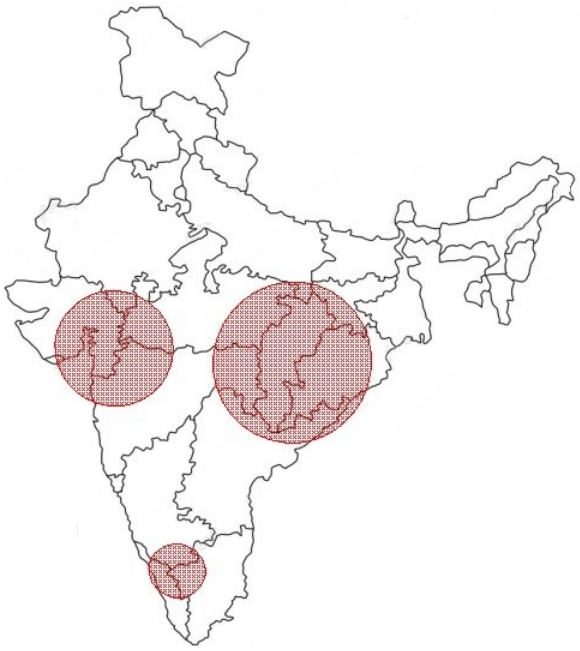
He studied in following institutions for different projects in the field of SCD research as follows:

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.

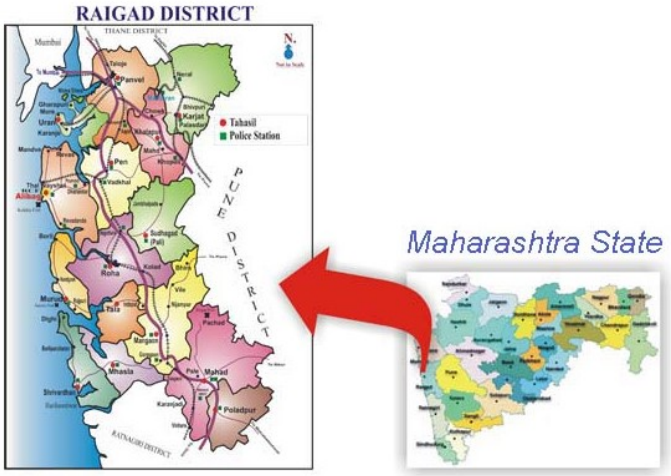


Fig. 1: Study location : Raigad district (Western zone; India)

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

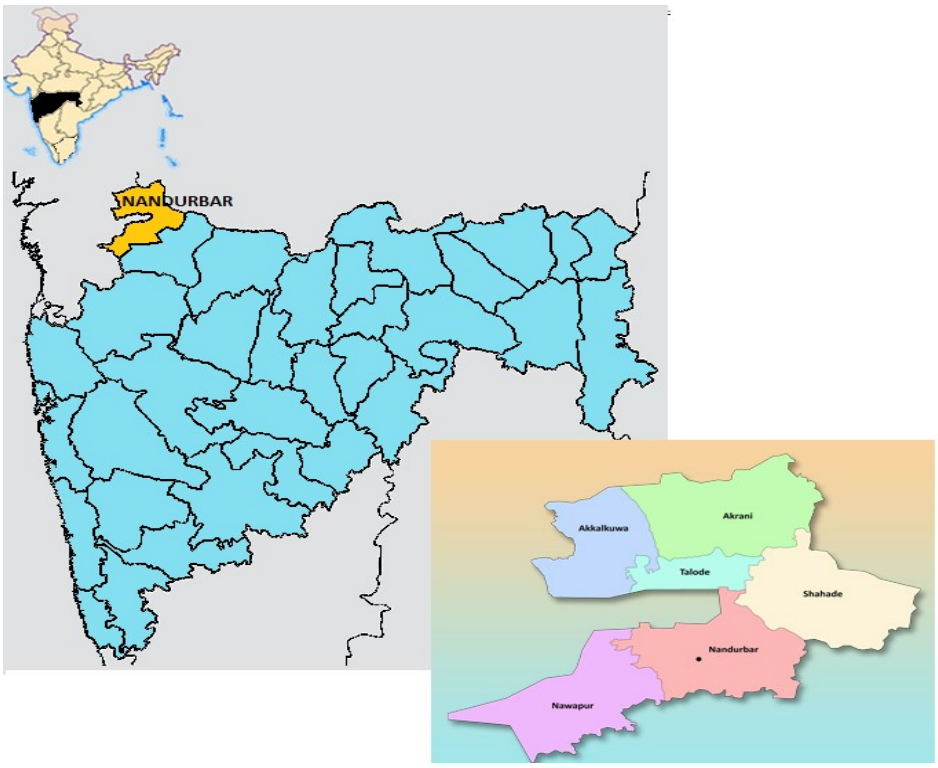


Fig. 3: Study location: Nandurbar district (Western zone; India)

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

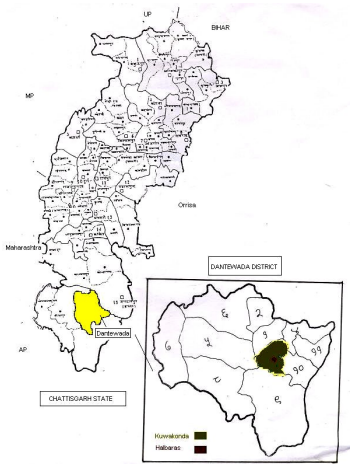


Fig. 4: Field work site, Bastar region, Chattisgarh state

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

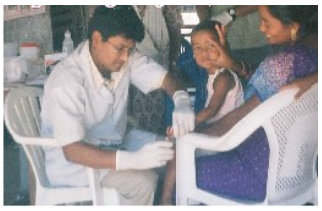


Fig. 6: Pediatric sampling

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

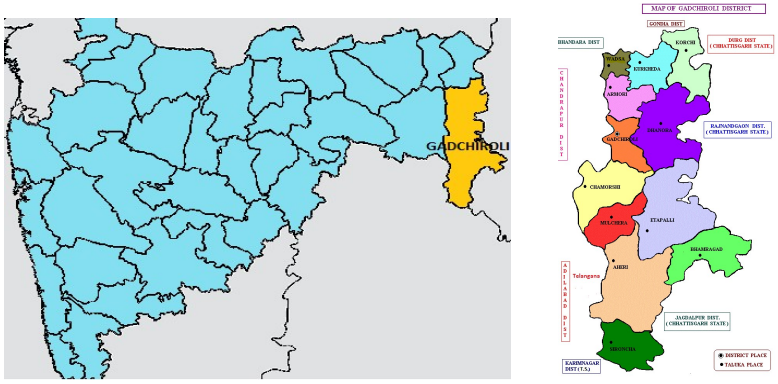


Fig. 10 Study location: Gadchiroli district (Central zone; India)

<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: Wayanad district (Southern zone; India)

Position: Principle Investigator
Duties and contribution: 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, 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. Trained all of them before field studies and laboratory work. We planned 32 visits, (villages and one OPD in the same lab at Ballarpur).

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.

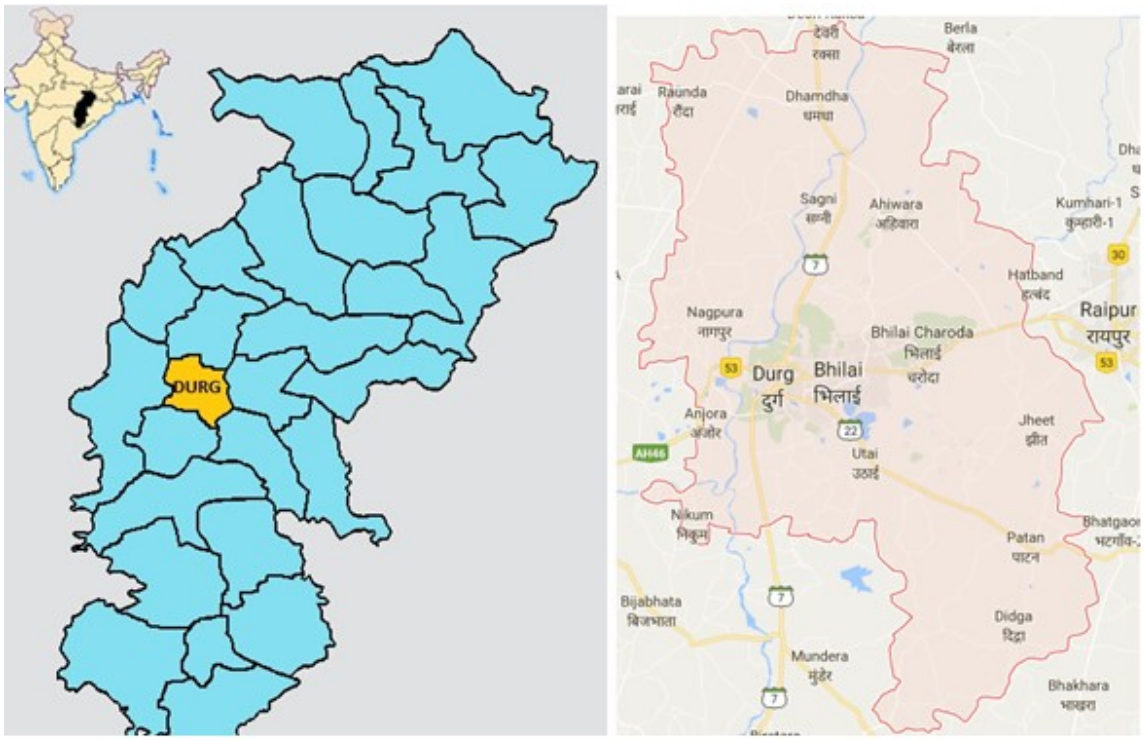


Fig. 21 Study location: Durg district (Central zone; India)

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.