Brief report on Sickle Cell Anemia studied by Dr. Devendra Lingojwar (1999 -2013)

This project report is all about sickle cell anemia prevalence studies, pathogenesis studies, and technology development in diagnosis from 1999 to till date. Some activities such as NGO establishment and publication preparation are also mentioned in brief. Total 8 projects, including short term field visits as well as funded projects, have been described in brief. Project no. 6, which was carried out in the state of Maharashtra which was one of the largest project on random sampling is described in details with reference to project writing, implementation, clinical studies. The first ICMR project, where I was employee is not considered under the list of own projects.

Year 1999: Appointment by ICMR, Training, Lab setup, Field visit and screening

Location: Western India, Maharashtra state, Raigad district, Karjat, Village: Neral

The first exposure of Sickle Cell Anemia 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 in 4 states (Tamilnadu, Maharashtra, Gujarat and Orissa) for clinical evaluation and screening for nutritional anemia, sickle cell disease and other hemoglobinopathies as well as G6PD deficient among primitive tribal populations in these regions. The prevalence of nutritional anemia and sickle cell trait and disease was established and intervention given to individuals with iron deficiency and sickle cell disease and these cases were followed up to evaluate the effectiveness of the intervention program.

Duties and contribution: One of the centers for sickle cell anemia was established at Neral, district Raigad Maharashtra and most of the sickle cell anemia prevalence studies were based on *Katkari* tribes of Raigad district. Major duties involved, to set up laboratory in tribal area including tracing of tribal school children from ashram schools for studies, laboratory set up from scratch, weekly collection of intravenous blood samples, processing at field station, Hemoglobin estimation, sickle cell anemia screening by solubility test method followed by confirmatory test on all samples by Cellulose acetate membrane electrophoresis, Blood group, Red cell enzymopathy test: G6PD deficiency test, Parent studies (i.e. Pedigree analysis) of tested patients and report preparation and submission to ICMR. Data on tests samples not available to me, on an average we studied 100 samples per months. (After excluding initial training duration at least 10 months dedicated to actual lab work, estimated sample size for sickle cell studied might be >1000).

Imp. Note: Data is not available and / or can not be given, as I was working as an employee of ICMR for this project. Please do not consider this as own project.

Fig. 1: Indian states with tribal population in %

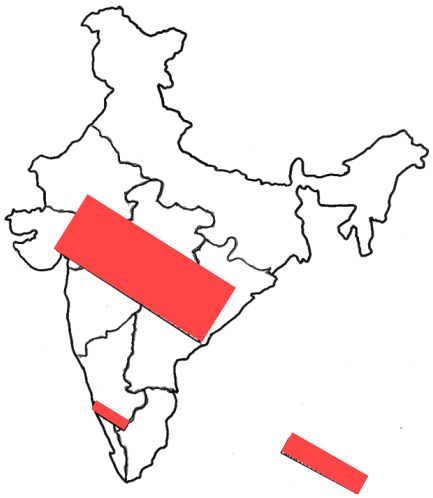

Fig. 2: Sickle cell anemia affected population in India

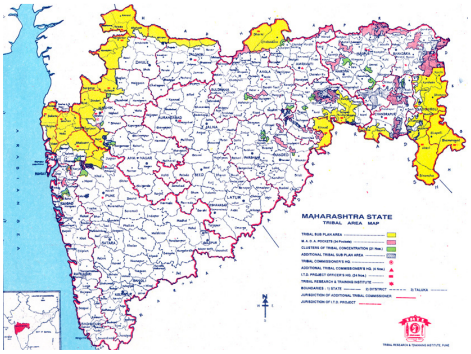

Fig. 4: Tribal region of Maharashtra state, region is yellow color in Maharastra state is under Tribal sub plan

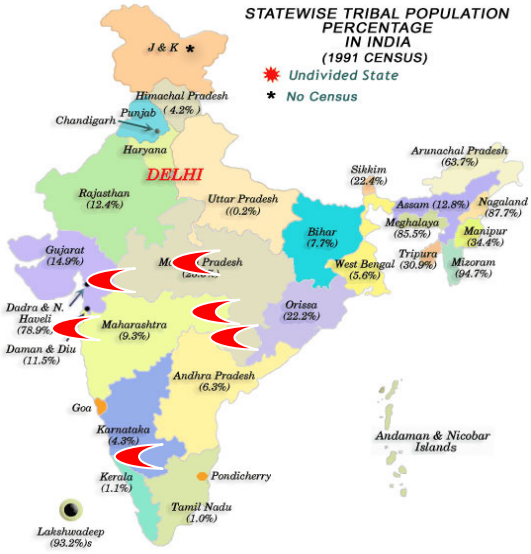

Fig. 3: Sickle cell anemia studies carried out in following states: Maharashtra, Chattisgarh, M.P and Keral.

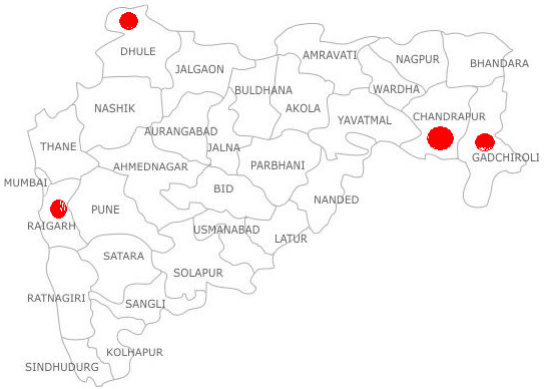

Fig. 5: Some of the Tribal region of Maharashtra state, studied since 1999. Red color indicates actual field work and lab studies: Chandrapur, Gadchiroli, Raigarh and Nandurbar

Sickle cell anemia 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

Total sample size >650 (Hb, solubility test, electrophoresis)

Imp. Note: Data is not available.

Year 2002

Establishment of NGO- Regional Society for Education and Research in Community Health (RESEARCH) Pune, Workshop 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 project 2:

Year 2003: Field visit (detail data are 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: MAM and RESEARCH Pune, MH

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 (sample collected in Nandurbar district, MH).

Sickle cell anemia project 3:

Year 2004: Field visit (detail data are available)

Chattisgarh state

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

Funding agency: MAM and RESEARCH Pune, MH, BKNS Halbaras, Chattisgarh

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 %

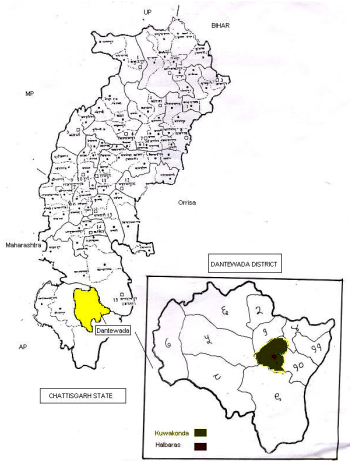

Fig. 6: Field work site, Bastar region, Chattisgarh state

Fig.7: Solubility test: Along With Hemoglobin electrophoresis, this is the golden standard for final laboratory detection of sickle cell anemia.

Fig. 8: With sickle cell anemia affected Families from Chattisgarh

Sickle cell anemia project 4:

Maharashtra state: Field visit (detail data are available)

Funding agency and manpower: RESEARCH Pune and Lions Club Ballarpur MH,

Chandrapur district: (13th to 15th September 2004) Ballarpur city, Total screened 129, Prevalence of sickle cell anemia = 17.82 %,

Gadchiroli district: 20th to 21st September 2004 at Kurkheda, Total screened 27, Prevalence of sickle cell anemia = 25.92%

Fig. 9: Counseling in first Sickle cell camp at Ballarpur

Important note: Some of new patterns of hemoglobin (total 4 samples) needs to work for different variations of the beta globin chain, need to examine based on molecular studies. Electrophoretic mobility of these four samples on cellulose acetate membrane at an alkaline pH of Tris Glycine buffer was different than that of hemoglobin S or hemoglobin D. Protein sequencing of these globin chains will be significant for finding variant of hemoglobin.

Sickle cell anemia project 5:

2005 Field visit (detail data are 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: RESEARCH Pune

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

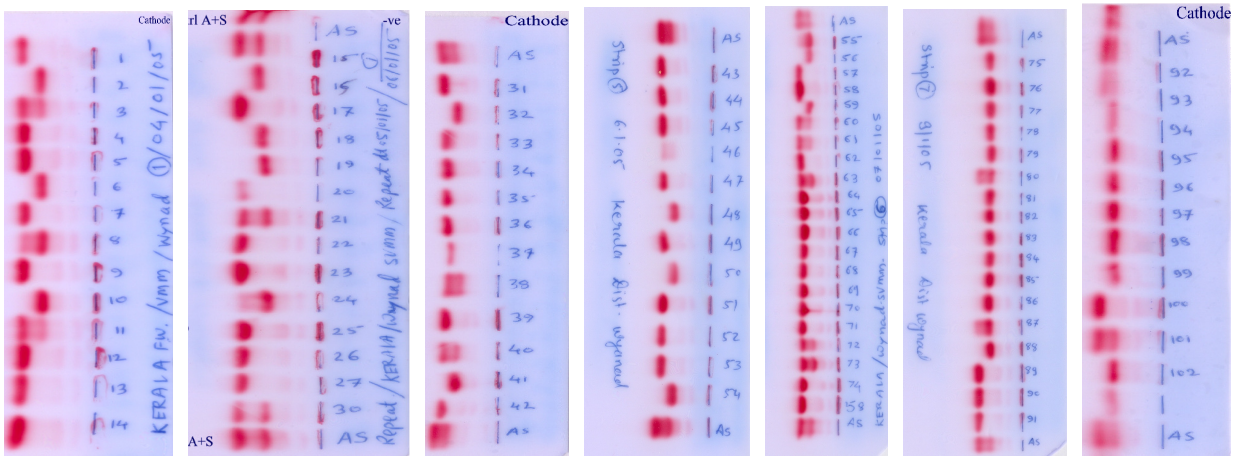

Fig. 10: Total studied sample visualized in confirmatory test results from Keral studies (Jan. 2005)

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 (detail data are 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: Christian Relief Society (CRS), Mumbai
Duration: project duration, 1 year (actual field and lab studies: 6 months)
Post: Principle Investigator

Duties and contribution: Based on initial 1 week project carried out in Ballarpur city during 2004, detail project was drafted and prepared and applied for funding,
After 1 year, received grant from CRS Mumbai. Recruited lab technician, MSW, devoted college students, along with ambulance for field work. Field and laboratory studies. Medical professional (MBBS) with assistant doctors were also recruited for clinical examination and blood collection. Trained all of them before field studies and laboratory work. We planned 30 visits to 15 villages and one OPD in the same lab at Ballarpur.

Fig. 11: Inauguration of Sickle cell camp at Ballarpur by Dr. Devendra Lingojwar Founder President "RESEARCH"

Fig. 12: Sickle cell camp Information by Dr. Devendra Lingojwar During school screening program, Kalmana, Dist Chandrapur

Fig. 13: Sickle cell field work team, this dedicated team including lab technician, MSW, driver and volunteers worked in all 30 field visits

Out of 30 visits, 15 visits were planned for random blood sampling as well as blood sampling of school children. Next 15 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.14: Sickle cell field work: complete school screening program

Fig. 15: Sickle cell counseling Dr. Devendra Lingojwar

Fig.16: Sickle cell field work team, with Ambulance, sponsored by MP, Mr. Naresh Pugaliya (Indian National Congress)

Fig.17: Inaguration of Sickle cell field work by MLA, Mr. Sudhir Mungantiwar, (BJP)

Major outcome: Out of 4008 random population screening, 49 homozygous sickle cell anemia individuals are found, sickle cell carriers are 550, Compound heterozygous status (sickle cell anemia and beta thalassemia in single patient) was associated in 2 patients, Clinical examination for vaso-occlusive crisis and human parvovirus virus associated pathogenesis studied. More episodes of blood transfusions as well as crisis and pain episodes were found in SCD children below 10 years.

This was the first largest random survey in Chandrapur district at large scale covering 16 locations and 32 field visits including follow-up studies.

Number of the sickle cell gene affected Prevalence of sickle cell anemia = 13.72%
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
Positive solubility tests were studied for confirmatory test by Cellulose acetate membrane electrophoresis = 1734; Total carriers = 550; Total sufferers = 49
Sickle cell carrier prevalence = total carriers x 100 / total screened
i.e. 550 x 100 / 4008 = 13.72 %

Summary of clinical studies in sickle cell anemia patients
During study period of July 2005 to Dec. 2005, sickle cell anemia field screening test screened a total 4008 samples from Chandrapur district. Hemoglobin electrophoresis test performed on 1734 samples, which includes solubility test positive, blood samples from families of an index patient and from solubility test negative anemic individuals. Clinical investigation performed in follow up visits to all 156 villages from Ballarpur Taluka, Chandrapur district and an OPD center i.e. testing lab based at Ballarpur.
Conclusion: : Sickle cell anemia prevalence rate in studied region was found to be 13.72 %. As compared to earlier studies in Gadchiroli district, prevalence rate increased as compared to the expected number of carriers in the district. Possible reason, most of the individuals are from Schedule caste in this Taluka. As this study was based on random sampling and with 4008 total sickle cell anemia screening, this data will prove a benchmark study for future sickle cell studies in this district.

Sickle cell anemia project 7:

Year 4 (2006) – Field visit (detail data are available)

Maharashtra state-Sickle cell anemia field studies Pune: Sickle cell anemia camp at Pimpale Gurav, Pune, 19th -20th March 2006, Total screened 202 for anemia studies; Number of samples screened for sickle cell gene 102; Prevalence of sickle cell anemia = 0 %

Sickle cell anemia project 8: Technology development in SCA diagnosis

Year 2007 to 2008: Devised simple method of sickle cell anemia screening on mass scale with single blood drop (i.e. Least volume); Applied for process patent, simultaneously tested on known sickle cell patients. With the same technology, at present we are carrying out in routine screening and confirmatory sickle cell anemia test at ATG LAB Pune.
(Patent filed; examination report received March 2013)

Sickle cell anemia project 9: Hb E among tested sickle cell carriers in Durg, Chattisgarh

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

Only Sickle cell anemia related publication, patent, abstract, reports, articles etc.

Publication

1. Kate SL **Lingojwar DP**, "Epidemiology of Sickle cell disorder in the state of Maharashtra" International Journal of Human Genetics (2002) Sep; 2 (3) 161-167

Abstract Published in International conferences

1. **Lingojwar DP**, Kate SL, Gore MM, Basu A "***International Symposium on "Emerging Viral Infections: New Frontiers and Challenges"***At NIV (ICMR) Pune (11th -13th Oct 2004) "Prevalence of human parvovirus B19 in some tribal population groups from India"

2. **DP Lingojwar**, SL Kate "***II International Update on Sickle Cell Disease and Other Sickling Syndromes"***, (26th - 29th February 2004) Regional Hemoglobinopathies Detection and Management Center (RHDMC), Indira Gandhi Medical College (IGMC) Nagpur "Short protocols in hemoglobinopathies"

3. Kate SL, Basutkar, **Lingojwar DP** et al – JBS Haldane's "***International conference on Human genetics"***, Bhopal, Madya Pradesh, India (Dec 2000) "Sickle cell anemia screening and confirmatory tests"

Abstract Published in National conferences / published reports

1. **Lingojwar DP** Sickle cell Sanjeevani (Sep 2005), 8-9, Ballarpur, Maharashtra state, India "Sickle cell anemia: Epidemiology & pathogenesis"

2. Kate SL**, Lingojwar DP** Recent trends in Biotechnology, Guntur, AP 2004,"Sickle cell disease in the state of Maharashtra"

Other Publications for sickle cell anemia for social awareness of SCD

1. Lingojwar DP (2005) Booklets on sickle cell anemia in local language of Maharashtra state (in local language of the state, *Marathi*) for doctors and patients as a part of sickle cell awareness program "Sickle cell anemia" (2005)

2. Lingojwar DP (2004, 2005) Sickle cell anemia related articles / news in local newspapers in Vidarbha (Hitwada, Lokmat, Lokmat Samachar)

Under publication

Lingojwar DP, Kate SL, Mishra AC, Gore MM, Basu A. et al, "Human Parvovirus B19 infections in Some Tribal Populations in India with Homozygous Sickle Cell Disease." (NIV Pune)