SICKLE CELL DISEASE
AMONG TRIBALS OF ATTAPPADY

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Study conducted for

Integrated Rural Technology Centre, Palakkad

Supported by
Kerala Research Programme on Local Level Development (KRPLLD)

Acknowledgement

A study of this magnitude was made easy and enjoyable by the generous help that we received from diverse quarters. The least we can do is to humbly acknowledge the debt.

The first and foremost to be thanked is Dr. Prabhudas, Medical Officer of Pudur PHC, who took immense interest in our work and gave instantaneous support practically to the entire project. His participation guarantied big turnouts in many survey centers since he is greatly respected by all residing in that area including tribals. He also used all his contacts to make sure that our work would proceed smoothly. Dr. Rajesh of Community Health Center, Agali and Dr. Sajith of Sholayur PHC were helpful in making local arrangements in their respective panchayats.

Dr. KA Aisabi, Head of our Department gave top priority to this work and permitted the use of any facility in the department that we desired. We appreciate her deep concern and constant encouragement. We also wish to place on record our appreciation for the great help extended to us by Dr. K Baburaj and Dr. G Rajan by actually participating in the field surveys.

We thank the KRPLLD for funding this project. Their non bureaucratic approach and friendly approach, whenever we needed them, is deeply acknowledged.

Finally, we wish to thank the people of Attappady, particularly the tribals for co-operating with us. It is our hope that this study justifies the great faith that they have in us and would make some movement towards a better quality of life for at least some of them.

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INTRODUCTION
AND
BACKGROUND

Of all genetic disorders to which man is known to be liable, there is probably no other that presents a collection of problems and challenges quite comparable to sickle cell disease and related disorders, because of its extensive distribution, problem created by its chronicity, and its resistance to therapy. It is a genetic abnormality whose control and cure still elude clinicians, research workers, and social scientists. Despite major advances in our understanding of the molecular pathology, pathophysiology, control and management of the inherited disorders of hemoglobin, thousands of infants and children with these diseases are dying through lack of appropriate medical care. This problem will undoubtedly increase over the coming years because, as a result of a reduction in childhood mortality due to infection and malnutrition, more babies with hemoglobin disorders will survive to present for treatment, thus steadily expanding the population of patients on long term therapy. As a result of such demographic changes, the impact of these diseases is now being felt all over the Indian subcontinent and in many parts of Asia[1].

Inherited abnormalities of hemoglobin synthesis are among the most common genetic disorders of man. They are divided into two groups. Those characterized by structurally abnormal hemoglobin variants are called the *hemoglobinopathies*. Diseases in which one or more of the normal polypeptide chains of hemoglobin are synthesized at a lower rate are the *thalassemias*. HbS is the most common structurally abnormal hemoglobin, the other common ones being HbC, HbD, HbE and HbO.

Sickle cell disorder refers to those states in which the red cell undergoes sickling when it is deoxygenated. The sickle cell diseases include those that produce prominent clinical manifestations as seen in sickle cell anemia, sickle cell HbC disease, sickle cell â-thalassaemia, sickle cell HbD disease etc. The term sickle cell anemia is reserved for homozygous state for sickle cell gene while sickle cell trait, which has never been considered a disease, has one abnormal gene[2].

History: The story of our ever growing knowledge of these conditions really began at the turn of this century. Long before it came to the notice of modern science, sickle cell anemia was known in Africa by different names all alluding to the recurrent and painful symptoms during the crises. The scientific investigation of the disease was set in motion by James Herrick in 1910. He reported "peculiar, elongated, sickle-shaped red corpuscles" in "a case of severe anemia" in a black student. The sickle cells, he thought were freakish poikilocytes and with considerable foresight, suggested that they were a manifestation of a peculiar chemical or physical condition[3]. Emmel, in 1917 observed the transformation of biconcave red cells to the sickle form in vitro. He also noted that sickling occurred in persons with severe anemia and in others who were apparently healthy, thus recognizing both sickle cell anemia and sickle cell trait[4]. Hahn and Gillespie in 1927 showed that exclusion of oxygen was prerequisite to sickling and that it could be reversed by re-exposure to the gas. They postulated that within the body, hypoxia leads to cellular distortion and hemolysis[5].

In 1940, Sherman observed that the cells in sickle cell anemia were birefringent[6]. Linus Pauling suggested that the birefringence could be due to the interaction between abnormal hemoglobin molecules. Pauling, Itano and colleagues at the California Institute of Technology demonstrated this by the technique of electrophoresis, in 1949[7]. They showed that the patients with sickle cell anemia had abnormal hemoglobin (named hemoglobin S) which could be separated from normal

hemoglobin (named hemoglobin A). In the same year, Neel established that sickle cell trait was the heterozygous and sickle cell anemia the homozygous state for the same gene[8].

Nomenclature: Since 1949, biochemists have discovered numerous other hemoglobins besides the sickle cell one. When newly discovered hemoglobins were first reported, they were designated by letters of the alphabet. Normal adult hemoglobin and fetal hemoglobin were called HbA and HbF respectively. When sickle cell hemoglobin was discovered, some called it HbB, but soon the letter S was assigned to it, and, to avoid confusion, no other hemoglobin was designated as HbB[9]. Other hemoglobins were assigned letters in alphabetical order. By the time the letter Q was reached, it had become apparent that this system would not provide enough designations.

Meanwhile it soon became clear that, since the various abnormal hemoglobins differ in electric charge, they must differ somehow in arrangement of amino acids in the peptide chain, for the amino acid make-up is responsible for the charge of the molecule. It was left to Vernon Martin Ingram to solve this problem. By paper electrophoresis and peptide analysis they showed that hemoglobin A, hemoglobin S, and hemoglobin C all differed only by a single amino acid. While the sixth position from the N terminal of the beta chain was occupied by glutamic acid in hemoglobin A, it was valine in hemoglobin S and lysine in hemoglobin C[10]. What was surprising was that, the small differences could account for dramatic symptoms. The concept of molecular disease was born with this discovery.

Abnormal hemoglobins are now assigned both a common name and a scientific designation. The common name is selected by the discoverer which usually represents a geographic area, such as a city, district, province, or hospital, and may be the native region of the propositus or the place of discovery. Scientific designations

indicate the variant chain, both the sequential and helical number of the aberrant amino acid, and the nature of the substitution. Thus, â6 (A3) glu val, the scientific designation of HbS, indicates that the substitution of valine for glumatic acid has occurred at amino acid number six, the third amino acid in the A helix, in the â-chain. Current recommendation calls for the use of solitary letter designations only in the case of normal hemoglobins HbA and HbF and abnormal hemoglobins HbS, HbC, HbE and HbH[11].

A catalog of human hemoglobin variants was maintained until 1998 by Dr. Titus H. J. Huisman. After his death, this syllabus was converted to an electronic database, which is expanded and revised on an ongoing basis[12]. It is accessible on the World Wide Web at http://globin.cse.psu.edn. As of now, the database includes no fewer than 850 unique hemoglobin variants.

Prevalence and Geographical Distribution: Frequency of abnormal hemoglobins varies with geographical locations and racial group. Four among the large number of abnormal hemoglobins described, i.e. S, C, E, and D Punjab, are called the "common abnormal hemoglobins" due to the fact that each of them affects millions of individuals[13].

Hemoglobin S is the most common of the abnormal hemoglobins and, consequently, sickle cell anemia the most common hemoglobinopathy. It is found in equatorial Africa in a broad belt extending from coast to coast. The highest incidence occurs in the eastern part of the continent, where about 40 to 50% of the members in certain tribes harbour the gene. A prevalence rate of 10 to 20% is common elsewhere in the belt[13]. The incidence of sickle cell gene in North and South American populations reflect their African origin. The overall prevalence of sickle cell disease among blacks in United States is 8%. The HbS gene is also found in non African populations. A prevalence of 25% has been reported in southern Turkey, Saudi Arabia

and in Israeli Arabs. HbS has also been reported with high frequency from the Northern Mediterranean shore, especially Sicily, Cyprus and Greece[13].

HbC is found in western Africa and also among blacks in the new world to a lesser extent. HbE has its highest prevalence in South East Asia. It is also seen in the North eastern states of India. HbD Punjab is primarily seen in inhabitants and migrants from Punjab and North western regions of India and Pakistan.

Apart from the above mentioned countries, sickle cell gene has been described in the aboriginal people of India. HbS has been found mostly in the aboriginal tribes in Nilgiri Hills, Central India, Orissa, Bihar, Madhya Pradesh, Gujarat, Karnataka and Kerala. HbD is found amongst Punjabis, Gujarathis and Bengalis; HbE in Bengalis and Assamese. A few instances of HbH, HbJ, HbK, HbL, and HbM have also been reported. Sickle cell–thalassemia has been reported from some centers. The different studies from India have been collected and published by the Indian Council of Medical Research[14]. In Kerala, a high frequency of sickle cell gene has been reported among the tribal communities of Wyanad and non tribal Wyanadan Chetti by us and another group from AIIMS, New Delhi[15,16].

Tribal situation in Attappady: The Attappady valley is a southward extension of the Gudalur plateau of the Nilgiris, east of the waterland line of Western Ghats sloping towards the east. It is at an average elevation of 800-1000 meters with the needle shaped Malleeswaran peak (appr. 2000 meters) as its most notable landmark. The Bhavani and the Siruvani rivers flow south and join outside the limits of Kerala[17].

The valley is inhabited by tribals belonging to three tribal communities. The predominant of them is the Irula community followed in number by the Muduga and the Kurumba communities respectively. The population of each of these communities according to the 1991 census and the projected population for the year 2001 are given

below. The projection is done by using the available data of the growth rate between 1981 and 1991.

Table 1-1: Population of the tribal communities in Attappady

Community	Population (1991)	Projected pop.(2001)	Growth rate	% of ST pop. in Kerala	% of ST pop. in Attappady
Irula	21,836	25,502	16.79	6.8	77.7
Kurumba	1820	2581	41.86	0.6	7.9
Muduga	3762	4740	26	1.1	14.4

Irula: The most numerous of the three tribal communities in Attappady valley, Irulas are distributed among 106 hamlets in the eastern half of the valley comprising Agali, Pudur and Sholayur panchayats. Like other tribal hamlets in the valley, the Irula hamlets are also sited in such a way that they command a view of the Malleeswaran peak[17]. The name Irula means dark or blackness (irul), whether in reference to dark jungles in which they reside or to the darkness of the skin, is doubtful[18].

Irula practiced shifting cultivation on the forested uplands, dry land farming with ploughing and wet land paddy cultivation. They are expert collectors of honey from the beehives. As more and more of them got educated, the proportion of those taking tertiary occupations under Government departments is rising. They are patrilineal and patriarchal. Monogamy is the norm. Polygyny is sanctioned but polyandry is strictly forbidden. The literacy has increased from 11.75% in 1981 to 29.3% in 1991. They consider Kurumba as their superior and Muduga as equal, even though the Muduga consider the Irula as inferior. The Kurumba and Muduga inter-marry but the Irula are not accepted.

They will not eat flesh of buffalos or cattle, but will eat sheep and goat, field-rats, fowls, deer and pig[18].

Kurumba: Etymologically, Kurumba means one who tends sheep. In Kerala, they are concentrated in Attappady. Kurumbas of Attappady are called 'Palu Kurumbas' to distinguish them from the 'Alu Kurumbas' of the Nilgiris. They seem to be two segments of the same tribe, differentiated from each other because of the territorial separation.

They were semi-nomadic in the past and continue to abandon old sites for new. Anavayi, which was one of the survey centers of the present study, is considered by them as their original settlement. They form the least number of the three communities. They had a unique system of shifting cultivation described as 'dig and scratch' method by which they would just dig enough to dabble the seeds or plant the seedlings. Roots and tubers are collected throughout the year. They are fond of honey. According to the tradition they would consume half of what they collect and exchange the rest.

They are patrilocal and patrilineal. Polyandry is forbidden, but polygyny is a status symbol. They have a well established tradition of herbal medicine associated with magico-religious practices for curing many diseases[19]. They also use modern medicine and show a favourable attitude towards family planning programmes[20].

The culture and social organization of Kurumbas despite the presence of shifting cultivation, herding, wage labour, marketing etc. are in many ways more like those of hunter-gatherers than of horticulturists. Thus, they can be labeled as a settled foraging society with a social organization similar to a delayed-return system like the native Australian and Andaman islanders[21].

Muduga: They are tribes settled around the Malleeswaran peak in Attappady. This community is confused with Muduva, an entirely separate community living in Idikki.

Census reports have considered both as one with a population of 11,203 in 1981. The population of Muduga living in Attappady region is only 2,370 in 1971[22].

Muduga are believed to be the earliest immigrants. They consider themselves equal to Kurumba with whom they inter-marry. They feel that Irula are inferior and do not accept food from them. They were shifting cultivators and have now become largely landless.

Although Irula are considered to be the most inferior tribe, they have caught up with the other tribes in literacy rates, particularly the female literacy, and sex ratios; although literacy rates are dismally poor across the board. The data available for 1991 are given in the table below.

Table 1-2: Literacy rates of tribal communities in Attappady

Community	Sex ratio (for 1000 males)	Literacy %	Female literacy %
Irula	988	29.3	25.7
Kurumba	906	22.6	15.2
Muduga	983	24.3	17.1

Sickle Cell Anemia – Disease Characteristics: The symptoms and signs of sickle cell anemia are due to the abnormal shape assumed by the red cells when exposed to lowered oxygen tension. These assume mainly two forms. Such cells get more rapidly destroyed and get removed from the circulation resulting in anemia. More importantly, the abnormally shaped cells increase the viscosity of blood and cause blockage of small blood vessels. The clinical features of sickle cell anemia result more from the vaso-occlusive consequences of sickle cells than from the anemia itself. They may be divided into those that characteristically are acute and episodic, and those that are chronic and often progressive. The acute episodes result in acute

painful episodes, pertaining to different organs and are known as crises. Blockage of vessels and consequent micro-infarcts in the bone cause the bone and joint crises; which is characterized by exquisite pain. Similar events occur in the mesentery and abdominal viscera, especially the spleen. Vaso-occulsive events in the central nervous system may be catastrophic. Stroke due to occlusion of major cerebral vessels and intracerebral or subarachnoid hemorrhages are the main central nervous system syndromes that occur in sickle cell anemia. Acute chest syndrome characterized by chest pain, fever and tachycardia are said to be the single most important cause of hospitalization in sickle cell anemia. This syndrome is due to pulmonary vaso-occulsion and infection.

These acute vaso-occlusive events are popularly referred to as sickle cell crises. According to Diggs, who defined sickle cell crises in 1965, the word crises (greek Krisis, turning point) is defined as a sharp turn or definite change in the course of a disease, with the development of new symptoms and signs[23]. By usage the term implies alteration that is unpredictable, often suddenly, and affects the patient adversely. The term "sickle cell crises" refer to any new syndrome that develop rapidly in patients with sickle cell disease due to the inherited abnormality and is not explainable on any other basis. Most common precipitating cause is respiratory infection. Typhoid fever, specific and nonspecific enterocolitis, otitis media and meningitis are other infectious offenders[24]. These infections, together with their pathophysiologic effects like fever, dehydration and acidosis create conditions that invite sickle cell crises as seasonal factors. The highest admission peaks of patients in crises are related to the highest rainfall months. However no seasonal variation has been noted by Diggs and Flowers[25].

The crises may be considered under two main headings (1) those due to vascular occlusion without major hematological changes and (2) those due to imbalance between blood formation and blood destruction. The first group is a

variety of syndromes which are typically recurrent and potentially catastrophic. Clinical manifestations are sudden in onset and directly attributable to obstruction of the microcirculation by intravascular sickling. Precipitating factors are, however, rarely identified in adults.

Acute illness also results from sudden worsening of anemia as mentioned before. This in turn may be a result of infections, sudden cessation of red cell production (aplastic crises), folate depletion (megaloblastic crises), or sudden increase in the quantum of hemolysis (hemolytic crises) [26].

Patients with sickle cell anemia are more prone to certain types of infections. During the first 5 years of life there is a definite increase in Streptococcus pneumoniae infections[27]. Later this is replaced by gram negative infections, especially those by Salmonella and Hemophilus influenzae. Indeed Salmonella osteomyelitis is characteristic of sickle cell anemia. This peculiar pattern of infection is related to the loss of splenic function consequent to the involution of the spleen, as a result of repeated infarctions (autosplenectomy) [26].

Sickle cell anemia also causes chronic morbidity and disablement. Stunting of growth may occur at different sites as a result of repeated vaso-occulsive episodes; rarefaction of bone, chronic joint disease, and chronic lung disease characterized by decreased radiolucency and impaired pulmonary function. The cardiovascular system is stressed by chronic anemia, recurrent small pulmonary artery occlusions, and myocardial hemosiderosis. Liver enlargement and recurrent jaundice are extremely common and gall stones occur eventually in a majority of patients. Nodular cirrhosis may be found in some adult patients. Kidney involvement in sickle cell anemia is manifested by a loss in concentrating capacity resulting in hyposthenuria and decreased capacity of hydrogen ion secretion. Papillary necrosis and nephritic syndromes primarily due to membranoproliferative glomerulonephritis are rarer renal complications. Proliferative and non proliferative retinopathies are among the

different ways in which the eye can be affected in sickle cell anemia. Chronic leg ulcers are another distressing manifestation of the disease in some patients. Pregnancy and surgery sometimes precipitate crises or other complications[26].

Mentioned above is a non exhaustive list of the clinical manifestations of sickle cell anemia. Even then it can be readily appreciated that it can present in myriads of ways and that it can mimic different other diseases. Strong awareness of the possibility of sickle cell anemia and ready means of laboratory diagnosis are essential in areas of high gene prevalence. Sickling test and different solubility tests are helpful in screening for the gene. They cannot however differentiate between homozygotes having disease and asymptomatic heterozygotes. Hemoglobin electrophoresis is valuable for this differentiation as also for detection of other abnormal hemoglobins.

Variability of Sickle Cell Disease: Sickle cell anemia is remarkably variable in its clinical expression. If these phenomena could be quantified, perhaps by a severity index of symptoms or by the age of the surviving affected individuals, it is likely that they would follow a normal distribution curve. At one end of this curve would be the children with repeated severe crises and death prior to the age of 10 years from septicaemia in aplastic crises; and at the other end would be those patients who without much trouble might reach adulthood and might even serve unrecognized in the armed service for several months before they became symptomatic. Diagnosis would finally be made by an alert physician or a more alert laboratory technician.

That, clinical variability is related to as yet undefined genetic variables is suggested by the tendency for disease severity to follow selected geographic and ethnic lines. It is found to be severe in Africa and African immigrants in US. Mild disease is the feature in Turkey, Greece, Cyprus and India[28,29]. The disease in Wyanad has also been shown to be mild[15]. The diversity is classically demonstrated in Saudi

Arabia where both mild and severe forms co-exist. Sickle cell disease prevalent among natives residing in Eastern province of Saudi Arabia have a mild phenotype while those with sickle cell gene in the South West province, who have migrated from central Africa, have very severe disease[30]. Number of sickle cell crises per year, hospitalization and requirement for blood transfusion were more than double in severe cases compared to the milder form. The disease in Senegal (Atlantic West Africa) and Benin (central West Africa) differ in several hematologic characteristics[26]. Patients with apparently mild sickle cell anemia are found on further study to be doubly heterozygous for HbS and another beta chain abnormality like alpha or beta thallassemia or another hemoglobin with the same electrophoretic mobility as HbS. Further, the sickle cell mutation may have occurred more than once in the human population and there may be as yet unknown but important linkage differences between these. Studies of the polymorphisms using the restriction endonuclease Hpa 1 adjacent to the beta globin site reveal three major patterns in human populations. A 13.6 kilobase fragment is associated with the mutation which arose in Western Africa. Elsewhere in Africa and in Asia including India it is associated with a 7.6 kilobase fragment[31].

Because Hb F is excluded from the Hb S polymer, it stands to reason that individuals with relatively more Hb F should have less severe disease than those with less Hb F. Support for such relationship is found in the eastern province of Saudi Arabia[32], Kuwait[33], Iran[34], and India[15,29], where mild disease is associated with HbF levels of 15 to 30%. The reason for the increased levels of HbF in the mild cases is not only because the cells bearing high concentration of HbF resist destruction, but there is also some evidence of an absolute increase in the synthetic rates of HbF. The reason for this is not clear. One possibility that is suggested is the presence in these individuals of the genes for Hereditary Persistence of Fetal Hemoglobin (HPFH). But other factors may also operate. It may also be noted that later studies in Saudi Arabia

revealed mild sickle cell anemia in the absence of elevated levels of HbF[30]. Increased HbF, instead of being protective may thus be just one of the phenotypic characteristics of the 'mild genotype'.

The existence of a relatively benign group of cases of homozygous sickle cell anemia in Jamaica has been documented[35]. The clinical and hematological features of the elderly group (over 30 years) were compared with those patients under the age of 30 years. The eldest patient in this study was 62 years old. There were no obvious differences in hematologic features, but the decreasing incidence of active leg ulceration and painful crises in older age groups suggested an amelioration of the disease symptoms with age. The possible genetic and environmental factors that promote survival of sickle cell anemia patients in Jamaica suggested were (1) high mean temperature and small annual temperature variation which decreases the peripheral vasoconstrictive phenomena (2) altitude: the concentration of population is around the coast at sea level and residence over 2,000 feet is unusual (3) eradication of falciparum malaria.

Malaria and Sickle Cell Disease: Prior to present century, the majority of individuals with sickle cell anemia almost certainly died before the age of reproduction. Unless individuals with sickle cell trait possessed some relative advantage, the gene for sickle hemoglobin would gradually be eliminated from the population. Allison in 1954 has suggested that an increased resistance to infection with malaria may be such an advantage[36]. Recognition that sickle cell trait has its highest prevalence in areas hyperendemic for malaria suggested that Hb S afforded a selective protection against lethal forms of malaria. Only the presence of certain abnormal hemoglobins, notably HbS, is capable of limiting the intracellular growth and intense parasitemia[37]. The tactoids formed during sickling of red cells may directly damage the parasite and render the deformed erythrocytes more susceptible to

phagocytosis. A similar protective effect may be exerted in thalassemia, G-6-PD deficiency or pyridoxal-kinase deficiency, since these abnormalities are found more commonly in malarious areas. The protection may be related in part to the persistent production of HbF since maturation of Plasmodium falciparum is retarded in cells containing HbF and, in part, to the increased susceptibility of such erythrocytes to oxidant change.

However, the wrong impression created in the literature over the years that sicklers are resistant to malaria, needs urgent correction. Rafer in 1955 studied 1,200 children attending hospital with Plasmodium falciparum and found that the parasite counts were much lower in sickling than in non-sickling children. It is maintained that the presence of HbS in red blood cells does not prevent, but limits the severity of Plasmodium falciparum infection in non-immune subjects. This also means that probably the attacks will be fewer. Thompson in 1962 confirmed this protective effect in children in Ghana. But he found that among the 278 adult police men reporting sick with fever in Accra district, Ghana, the incidence of positive blood films was highest in those with sickle cell tract and lowest in those with HbC trait[38]. Normal population showed incidence in between the above two groups. These findings suggested the theory that HbC is the result of a favorable mutation at the genetic locus responsible for HbA and HbS, and suggest that the protective effect exerted against malaria by HbS is due to sickling of parasited erythrocytes and is accompanied by symptoms.

Screening Programmes: When a condition threatens not just the comfort but even the lives of a specific population as well as of future generations, concerted and vigorous efforts are required to reduce the toll, whether the population proportions are 5% or 40%.

Screening programmes for the detection of HbS gained popularity in the 1970s. The goal of these programmes initially was uncertain. Because much of the screening was not coupled with effective education of those screened, there was widespread misunderstanding about the critical difference between sickle cell anemia and trait. The "stigma" of having HbS was reinforced with inappropriate health, social, and economic restraints. Against this background, guidelines for genetic screening were developed and a number of properly designed, comprehensive screening programmes emerged[39].

On the opposite end of those who say, "Electrophoresis should be for all", are those who say "why screen for a disease for which there is no cure?" The answer is, "True, there is no cure for this malady, but it can be prevented, and genetic counseling is currently the only approach". Symptoms can be ameliorated by proper supervision. There is always hope for the development of other solutions in the future. If and when effective treatment becomes available, its prompt wide introduction also requires knowledge of the frequency of sickle cell disease in specific geographic areas so that treatment programmes will be directed to those areas within a country where frequency is highest, lest much effort be dissipated needlessly and ineffectively at great cost.

The purpose of screening therefore is three fold:

1. Case finding: The child with Hb SS must be identified as early as possible, and the patient with mixed hemoglobinopathy must be discovered at any age. The data thus obtained from screening in itself serve a dual purpose. (a) as a means of identifying the patient who needs care and (b) as a source of valuable data toward determining the incidence and prevalence of the disease in various sub groups of the population.
2. Counseling: Pinpointing of the patient with Hb AS allows him to obtain counseling, a procedure with two goals (a) genetic counseling to decrease the incidence of Hb SS by limiting productive mating between two heterozygotes and (b) advice as to

vocation or avocation and instruction to the heterozygote as to what environmental conditions might precipitate sickling.

3. Establishment of a registry: This would provide for the listing and identification of all patients with Hb SS and allow ready professional exchange of information.

One of the current screening programmes in New York City is conducted by FRESD (Foundation for Research and Education in Sickle cell Disease). They conduct their programmes in public schools or in community groups usually sponsored by organizations like block association, church groups, community centers and others.

The technique chosen for screening should be genetically diagnostic and should clearly differentiate between sickle cell anemia and trait and those disorders of hemoglobin having implications for health.

Preventive measures: Until a safe and widely applicable mechanism for the prevention of intravascular sickling is found, a high priority must be placed on the prevention of complications. Because vaso-occlusive crises are precipitated by infections, fever, dehydration, acidosis, hypoxemia and cold exposure, measures to prevent or remedy these conditions assume importance[40].

Treatment: In addition to prophylactic measures aimed at preventing specific complications of sickle cell disease, three treatment options are used: chronic blood transfusions, hydroxyurea and stem cell transplantation. Gene therapy remains a future goal. But it is the prophylactic measures that have really helped to increase the survival among homozygotes. Greater access to the effective primary health care and its proper utilization by the communities improves survival and quality of life of the affected people[15]. The simple adoption of a standardized protocol for the

management of febrile illnesses, correcting dehydration and acidosis can go a long way in management of sickle cell disease.

Prognosis and survival: Though the first four cases of sickle cell anemia to be described were all over 20 years of age, the emphasis rapidly shifted to this being a disease with greatest importance in childhood. The natural history of the disease seemed to vary with the environment, but a particularly bad prognosis was observed in Africa[35]. As recently as 1955, only two cases of sickle cell anemia had been reported in adults in Belgian Congo, despite the presence of the heterozygous state in 25% of the population[26]. As late as 1970, 50% of the children with sickle cell anemia in Zambia died before the age of three years[41]. In a study in western Uganda where the sickling rate was 35%, no case of sickle cell anemia was found[42]. In a retrospective review of autopsy cases from the United States, 20 to 30% of the cases were less than 5 years of age. The median age of death was 14 years and survival beyond 40 years was unusual[43]. The disproportionate number of deaths occurring in early childhood in these and other studies were the result of overwhelming bacterial infection and splenic sequestration crisis[26].

Better prognosis and survival in sickle cell anemia is seen in two situations. One is in the genuinely mild cases as seen in Saudi Arabia. The other is in areas where there have been great improvements in the health delivery. In the United States, prognostic expectation for patients with sickle cell anemia has undergone dramatic changes as a result of early diagnosis, patient education, and therapeutic interventions. Newborn screening for sickle cell disease and greater awareness of the unique needs of infants and children is effecting a change in prognosis. The simple adoption of a standardized protocol for the management of febrile illnesses reduced the cumulative mortality in a clinic population to 7% at 2 years and 13% at 3 years of age[44]. The Cooperative Study of Sickle Cell Disease documented an 85% survival rate at 20 years of age[26].

Background to the present study: The medical college hospital at Calicut caters to the six northern districts of Kerala. More than 48% of the tribals of the state live in Wayanad and Attappady. Observation of sporadic cases of sickle cell anemia in the hospital and the fact that all the cases were either from Wayanad or Attappady region led to carrying out a pilot study in those areas by us.

The pilot studies were based on sickling test, which picks all individuals with the sickle cell gene, but fails to differentiate between the homozygotes with clinical disease and the heterozygotes who are asymptomatic. First a total of 1024 subjects were screened in Wayanad which included all major tribal communities and Wyanadan Chettis residing there. This was followed by another study in Attappady which tested 261 subjects. Only the predominant Irula community in the Attappady region was examined. The other two communities, the Kurumba and the Muduga were not included in the pilot study as they were numerically very small. The results are given in the tables below.

Table: 1-3: Pilot Survey in Wayanad

Community	**No. Tested**	**Sickling +ve**	**%**
Paniya	315	96	30.5
Kuruma	268	78	29.1
Adiyan	57	14	24.6
Kurichian	55	1	1.9
Kattunayakan	53	6	11.3
Urali	42	11	26.2
Others	12	1	8.3
Total tribal	**802**	**207**	**25.8**
Wayanadan Chetti	**126**	**49**	**38.9**
Settlers	96	0	0.0

Table 1-4: Sickling Survey in Attapady

Panchayat	No. Tested	Sickling +ve	%
Pudur	79	21	26.6
Sholayur	110	32	29.1
Agali	72	4	5.6
Total (All Irulas)	**261**	**57**	**21.8**

After the pilot study brought out the high frequency of the sickle cell gene for the first time in these areas, the critical role of identifying the homozygotes and heterozygotes accurately was recognized. It was not only important for the individualized management of the homozygotes but also crucial for the development of a comprehensive health care programme for sickle cell disease to be implemented in those areas. And the need of the hour was to conduct an Hb electrophoresis based study.

For the first time in Wayanad a Hb electrophoresis based study was conducted by us, supported by the State Committee on Science, Technology and Environment (STEC). It confirmed the results of our pilot study and also threw light on the frequency of homozygote cases in different communities and the severity of the disease in Wyanad. It helped in a big way to define specific recommendations for a comprehensive health care for sickle cell disease in Wayanad district[15].

The results are given in the tables below.

Table 1-5: Electrophoresis Patterns in Different Communities

Community	Total No. Tested (N)	Number			Percentage		
		AA	AS	SS	AA	AS	SS
Paniya	279	228	49	2	81.7	17.6	0.7
Kuruma	256	170	80	6	66.4	31.3	2.3
Adiya	91	62	25	4	68.1	27.5	4.4
Kattunayakan	52	50	2	0	96.2	3.8	0
Oorali	11	10	1	0	90.9	9.1	0
Kurichan	68	68	0	0	100	0	0
Others	2	2	0	0	100	0	0
Total tribal	**759**	**590**	**157**	**12**	**77.7**	**20.7**	**1.6**
Wayanadan Chetti	**214**	**141**	**62**	**11**	**65.9**	**29.0**	**5.1**
Tribe + Chetti	**973**	**731**	**219**	**23**	**75.1**	**22.5**	**2.4**
Settlers	21	21	0	0	100	0	0

The only data available on the prevalence of sickle cell disease in Attappady region is the pilot study we had conducted based on the sickling test. This is the only other area, other than Wyanad, where sickle cell gene is present in Kerala. Justice will be done to the tribals of Attappady, only if a study is conducted which can detect the frequency of sickle cell homozygotes and heteozygotes in all the three communities residing in the region and assess the severity of the disease there. This can help proper formulation of the strategy for the comprehensive sickle cell disease care in the region.

OBJECTIVES

1. To determine the sickle cell gene frequencies in the Irula, Kurumba and Muduga communities of Attapady.

2. To study the disease characteristics of sickle cell anaemia in those communities.

3. To calculate the deficit in survival of homozygous cases among tribals of Attapady, if any, and to compare the data with those in Wyanad and in other states.

4. To evolve a comprehensive health care system for the diagnosis and management of sickle cell disease in Attapady within the existing health care services available in that region.

MATERIALS
AND
METHODS

The survey: The survey was conducted in 15 separate centers spread over all three panchayats of the region. The help of the health service personnel and local leaders were sought for the mobilization of the tribals. Care was taken to mobilize all the subjects to attend the camps irrespective of whether they were healthy or sick. In the colonies and hamlets this was not a problem since all available members were examined. Similarly in the schools and tribal hostels all the children belonging to the tribal communities were examined. Even in one instance where the primary health centre was taken as the survey site, the selection bias was minimized by examining all the healthy bystanders also.

Selection of places for survey was not random in that the convenience of travel and contacts of the local organizers were taken into account. The selection of the individuals to be tested in each centre was however as unbiased as possible. Care was also taken to have adequate samples from all the three tribal communities.

Generally no difficulty was experienced in subject compliance. One reason for the general acceptance we feel, is because blood was collected by the finger prick method only and not by the conventional venepuncture. The list of centers where survey was conducted and the number of people tested in each center is given below.

Name, age, sex and community were noted for each person along with the clinical history of illnesses if any. History of recurrent jaundice, bone and joint pain, abdominal pain, leg ulcers were specifically asked for. Physical examination was

conducted particularly looking for growth retardation, anemia and hepatosplenomegaly.

Table 3-1: Survey centres

Sl. No.	Centre	Number
1	Cheerakadavu Irula Ooru	36
2	Bhoothivazhi Irula Ooru	70
3	Anavayi Kurumba Ooru	48
4	Mukkali Muduga Ooru	17
5	Varakampady Irula Ooru	20
6	Pudur Tribal Boys Hostel	91
7	Pudur Government High School	36
8	Pudur Primary Health Centre	121
9	Agali Tribal Girls Hostel	91
10	Sholayur Government High School	198

Blood Collection and Hemolysate Preparation: Blood was collected by a deep finger prick using a disposable lancet. The finger was lightly squeezed so as to elicit four to five large drops of blood, which is collected directly into a 4 ml test tube containing normal saline. One drop of blood was used to prepare a peripheral smear.

Preparation of hemolysate was done immediately after the camp. Red cells were washed thrice in normal saline by centrifuging at 3000 rpm for three minutes and pipetting the supernatant. After the last pipetting, 5 drops of hemolysate reagent were added to the cell button and shaken to obtain the hemolysate. In those cases where less amount of blood was obtained and consequently the cell button was small;

fewer drops of hemolysate reagent were used. The hemolysates were run in cellulose acetate strips at pH8.4. Hemoglobin electrophoresis was done within 24 hours.

Hemoglobin Electrophoresis: The method followed by us is briefly outlined.[16]

Equipment

1. Electrophoresis chamber and Power pack
2. Cellulose acetate membrane (Helena Biosciences)
3. Applicator
4. Wicks and blotting paper
5. Glass plate
6. Staining set-up

Reagents

1. Hemolysate reagent (Helena Biosciences)
2. Tris- EDTA-Boric acid buffer pH 8.4 (Helena Biosciences)
3. Working buffer: Stock buffer powder dissolved in 1000 ml. distilled water
4. Staining solution

Ponceau red	200 gm
Trichloroacetic acid	7.5 gm
Sulfosalicylic acid	7.5 gm
Distilled water	Upto 100 ml

5. Destaining solution

Glacial acetic acid	5 ml
Distilled water	100 ml

6. Clearing solution

Glacial acetic acid	30 ml
Methanol	70 ml
Clear aid (Helena Biosciences)	4 ml

Procedure:

1. Electrophoresis chamber is filled with working buffer. Whatman No. 3 filter paper is cut to suitable size and kept in each chamber serving as wick.
2. Cellulose acetate membrane is cut as per requirement. The membrane is dipped in buffer for 5 minutes and blotted between two pieces of Whatman filter paper quickly and evenly.
3. Hemolysate (1 to 1.5 microlitres) is applied with applicator on the mark of origin.
4. It is then placed on wicks, directing the mark of origin towards the cathodal end.
5. Glass plate is placed over it.
6. A current of 150 to 200 volts is applied for 30 minutes.
7. Power supply is switched off. The membrane is removed and stained with Ponceau red for five minutes. Excess dye is drained off and the membrane destained with 5% acetic acid till the background is white.
7. The strip is then put in clearing solution for 7-10 minutes and dried in incubator. The dried and transparent strips are then numbered and filed.

Hemolysates from known cases of sickle cell anemia served as controls for hemoglobin S. Cord blood was used as control for hemoglobin F. Normal blood had the HbA.

Peripheral smear examination: The peripheral smear was prepared, stained with Leishman's stain and screened in all the subjects particularly for hypochromic microcytic anemia. In all cases of sickle cell anemia the following points were specifically looked for: presence or absence of irreversibly sickled cells, target cells, anisopoikilocytosis, polychromasia and nucleated red cells. Irreversibly sickled

cells and target cells were assigned a grading of 3+ when they were present in almost all fields, 2+ when seen frequently and 1+ when only occasional cells were present.

Analysis: The essential data was entered in computer using an EXCEL programme. The results were analyzed with regard to gene frequency by age, sex, community, and region. All cases of sickle cell anemia were further analyzed to assess severity of disease, common symptoms, complications and laboratory findings. Cases of sickle cell anemia were categorized as severe when there was gross pallor and multiple episodes of crises. It was considered mild when pallor was clinically mild and if there was only one or no history suggestive of crises.

OBSERVATIONS

Of the 728 blood samples collected, 5 were unsatisfactory due to reasons like early hemolysis of the sample and breakage of test tubes during centrifugation. Hence the data presented is that of 723 persons in whom hemoglobin electrophoresis could be done satisfactorily. The age and sex characteristics of this sample are shown in the table below.

Table 4-1: Sample Characteristics

Age group	Male	Female	Total	%
0 – 10	74	71	145	20.1
11 – 20	168	223	391	54.1
21 – 30	26	78	104	14.4
31 – 40	14	10	24	3.3
41 – 50	8	9	17	2.4
51 – 60	7	13	20	2.7
> 60	10	12	22	3.0
Total	**307**	**416**	**723**	**100**
%	**42.5**	**57.5**	**100**	

The hemoglobin electrophoresis done on cellulose acetate at pH 8.4 in the 723 subjects revealed the prevalence of only hemoglobin S apart from the normal hemoglobins A, F, and A2. Other hemoglobins like hemoglobin D Punjab were ruled out because of the presence irreversibly sickled cells in the peripheral smear.

The results of hemoglobin electrophoresis in the different communities are given in the table below.

Table 4-2: Hb Electrophoresis Patterns In Different Communities

Community	Total No. tested (N)	Number			%		
		AA	AS	SS	AA	AS	SS
Irula	590	442	139	9	75	23.5	1.5
Kurumba	79	62	17	0	78.5	21.5	0
Muduga	45	34	10	1	75.6	22.2	2.2
Total	**714**	**538**	**166**	**10**	**75.4**	**23.2**	**1.4**
Settlers	9	9	0	0	100	0	0

The gene frequencies of the alleles A and S can be calculated from the hemoglobin electrophoresis data. The frequency of the A gene would be (AA + AS/2) / N and that of the S allele, (SS + AS/2) / N. Many studies on sickle cell gene, where only sickling test is done, give only the sickling positivity percentage which would correspond to AS + SS expressed as percentage of N. The gene frequencies and sickling positivity equivalent in this study is shown in the table below.

Table 4-3: A And S Gene frequencies in different communities

Community	Gene Frequency		Sickling Positivity Rate
	A	S	
Irula	0.867	0.133	25.0
Kurumba	0.892	0.108	21.5
Muduga	0.867	0.133	24.4
TOTAL	**0.870**	**0.130**	**24.6**

The distribution of AA, AS and SS according to the sex of the subjects is shown in the table below.

Table 4-4: Hb pattern by sex of the subjects

Sex	AA		AS		SS	
	N	%	N	%	N	%
Male	224	74.4	75	24.9	2	0.7
Female	315	76.3	90	21.8	8	1.9

The mean age of the sample is 18.4. The mean age according to hemoglobin pattern and sex is show in the table below.

Table 4-5: Mean age according to sex and Hb pattern

Hb pattern	Mean age		
	Male	Female	Total
AA	16.5 (7/12–75)	19.8 (8/12–85)	18.4
AS	19.8 (3½–70)	17.4 (1½–65)	18.4
SS	13 (12–14)	18.4 (15–26)	17.3
Total	**17.2**	**19.2**	**18.4**

The table below shows the mean age in different communities as per hemoglobin pattern.

Table 4-6: Mean Age according to Community and Hb Pattern

Hb Pattern	Mean Age		
	Irula	Kurumba	Muduga
AA	20.4 (8/12–85)	13 (2–60)	19.2 (2–60)
AS	18.8 (1½–65)	17.1 (5–37)	12.4 (10–18)
SS	17.2 (12–26)	–	18

The sample included persons from all the geographical regions of the district, as represented by the three panchayats of Pudur, Agali and Sholayur. The number of persons tested and their hemoglobin electrophoresis patterns are given in the table below.

Table 4-7: Geographical Distribution of the Sample

Panchayat	N	AS%	SS%	Sickling Positivity Rate
Pudur	309	25.6	1.3	26.9
Agali	177	18.1	0.6	18.7
Sholayur	229	24.0	2.2	26.2

Irulas were the most numerous and the most spread out community that was represented well in all the three panchayats. The electrophoresis patterns of Irula community from different panchayats are shown in the table below.

Table 4-8: Geographical Distribution of Irula tested

Panchayat	N	AS%	SS%	Sickling Positivity Rate
Pudur	321	24.3	1.2	25.5
Agali	70	17.1	0	17.1
Sholayur	225	24.4	2.2	26.7

There were 10 cases of sickle cell anemia. The table below gives the clinical and laboratory profile of these cases.

Table 4-9: Clinical and Laboratory Profile of SS cases

Sl No	Age	Sex	Comm-unity	Degree of Anemia	History of crises	Clinical Severity	ISC	Hypo-chromia
1	16	F	Irula	+	+	Severe	+	-
2	26	F	Irula	-	-	Mild	+	+
3	18	F	Irula	-	-	Mild	+	-
4	12	M	Irula	-	-	Mild	-	+
5	14	M	Irula	-	-	Mild	++	++
6	15	F	Irula	+	-	Mild	+	-
7	16	F	Irula	+	-	Mild	+	++
8	16	F	Irula	-	-	Mild	-	++
9	22	F	Irula	-	-	Mild	+++	-
10	18	F	Muduga	++	-	Severe	+	-

From the gene frequency of the hemoglobin alleles, the theoretical population distribution of heterozygotes and homozygotes for the A and S genes can be calculated using the Hardy – Weinberg equation. The square of the gene frequency of S multiplied by N, would give the expected number of sickle cell anemia cases or SS cases in the sample. This can be compared with the actual number of cases detected in the survey. This is shown in the table below.

Table 4-10: SS Cases – Expected and Actual

Community	**N**	**SS Homozygotes**	
		Expected	**Actual**
Irula	590	10.4	9
Kurumba	79	0.9	0
Muduga	45	0.8	1
Total	**714**	**12**	**10**

Presence of hypochromia in the red cells, which correlates well with the nutritional deficiency of iron, was looked into in all subjects. Moderate and severe hypochromia were taken as significant. The community wise distribution of hypochromic cases are given in the tables below.

Table 4-11: Prevalence of hypochromia in different communities

Community	**Moderate hypo.**	**Severe hypo.**	**Total**	**% of total tested**
Irula	59	15	74	12.5
Kurumba	9	3	12	15.2
Muduga	3	1	4	8.9
Total tribes	**71**	**19**	**90**	**12.6**

Of the 90 cases of hypochromic anemias picked up from the peripheral smears, 25 were from one survey site which was the Pudur Tribal Boys hostel, of which 16 were of severe form.

DISCUSSION

This is the first systematic, Hb electrophoresis based survey for detection of sickle cell disease to be conducted in the Attappady region, Palakkad, Kerala. A total of 723 subjects, from 10 different centers spread across the three panchayats of the region, were tested. Attappady was chosen for the study because Attappady is one area in Kerala where sickle cell gene is known to be present but has not been studied systematically. Also we are familiar with the place as a pilot study based on sickling test was conducted by us in this area before. Wayanad district, which is the only other area where sickle cell gene is present in Kerala, has been exhaustively studied by us and the results published. This gave us the necessary experience and confidence to conduct a study of such great dimension in a tribal area of very difficult terrain.

The survey: The active co-operation of the local orgnisations was sought for and obtained in the conduct of the survey. The key person behind the entire mobilization of the tribals to many of our survey centers was the Medical Officer of the Pudur primary health center. He not only participated very actively in most of our camps but also helped us find contacts in many other centers. The health service department with its efficient and effective network in the area was the mainstay of our organization of camps. The local panchayat members and other local leaders were also involved in the programmes. The tribal leaders of the various centers we visited took active interest in co-operating with us and mobilizing all the tribals in that locality. We found that the majority of the people had some access to Modern Medicine and

actively availed the services. And in no occasion did we encounter any kind of active resistance to the programme.

Subject selection and possibilities of bias: Care was taken to include all the three tribal communities residing in Attappady in the survey in proportions roughly corresponding to those found in the population. The actual sites of survey were fixed according to the contacts and convenience of the local organizers. In no instance was it influenced by any preconceived notions about disease or gene prevalence.

The centers set up for the survey were mainly of three types. They were the tribal hamlets and colonies, schools and hostels for tribal students, and in one instance a camp set up in the local Primary Health Centre. 5 out of the 10 survey centers were in tribal hamlets or colonies. All the persons present at the time, irrespective of whether they were ill or not, were tested. Attempt was made to notify the people in advance and timing adjusted for their convenience, but despite this, people going to work were sometimes missed. 4 centers were schools or tribal hostels. Here again, all the students of the tribal communities present on that day were tested. Of the 714 tribals tested 191 (26.2%) were tested at the place of residence, 416 (57.2%) in schools and hostels and 121 (16.6%) at the PHC.

It could be argued that patients with sickle cell anemia would be too ill to go to work and would be available for testing at home, whereas more of the normal people would be at work. While not denying the operation of this bias, we do not feel it is significantly high. For one, we were able to examine a high proportion of people regularly going for work. Moreover, as will be discussed subsequently, many cases of sickle cell anemia were of sufficiently mild nature so as to enable the affected persons to work. In the case of schools and hostels it could be argued that the diseased children would be frequent absentees or not enrolled at all and thus would be missed.

In the schools and particularly in the hostels, we talked to the teachers about the signs and symptoms of the disease and asked about any children having these. Again we do not think that the negative bias due to absenteeism is high. However, we have no idea regarding the bias due to non enrolment. In any case it is possible that both overestimation and underestimation of sickle cell anemia in case of colonies and schools respectively has occurred to a small extent. It is hoped that they would tend to cancel themselves out.

The age proportion of our sample is not truly reflective of the age structure of the population. We avoided testing children below the age of two, as far as possible, because of the problems of blood collection in a camp situation. Also, the school-going age groups are likely to be somewhat overrepresented because 57.2% of the survey population was from schools and hostels. This would cause some problems while projecting the data for the population at large, to obtain the numbers of sickle cell homozygotes in the whole population, provided most of the mortality in sickle cell anemia occurred at a very young age. We however have not found this to be the case as will be discussed in detail later.

The tendency to bring cases already diagnosed to have sickle cell anemia in hope of treatment is another source of possible bias. But since our camps were generally local neighborhood events, convened at short notice, this bias also did not operate to any significant extent. Of the 10 cases of sickle cell anemia diagnosed by us in the survey, none were previously diagnosed cases.

Blood collection and Electrophoresis: The standard method described in textbooks of laboratory hematology for hemoglobin electrophoresis is by collection of at least 2 ml of blood by venupuncture.[45] This method is however not suited for large scale field studies. One great success of the present study was that we could standardize the procedure for hemoglobin electrophoresis by the finger puncture technique. But for this, we do not think the camps would have been the great success

that they turned out to be. We generally obtained 4 to 5 large drops of blood from each patient. This was normally sufficient for the peripheral smear and hemoglobin electrophoresis. To obtain enough hemolysate it is necessary to ensure maximum cell button after the final wash. For this great care has to be taken to minimize loss of cells during washing process.

The hemoglobin electrophoresis was done on cellulose acetate paper at pH 8.4. We used the paper manufactured by Helena Biosciences, considered to be one of the best in the world. The excellent separation of the different hemoglobins was guaranteed once we decided to use of the buffer manufactured by the same firm. The dry paper had the additional advantage of enabling the strips to be stored after electrophoresis.

The hemoglobins D – Punjab that is found in Northwestern India, as well as the much rarer varieties like G – Georgia, G – Galveston, G – Philadelphia and Shimonoseki migrate to the same position as HbS on the cellulose membrane at pH 8.4[13]. However none of these hemoglobins are associated with symptoms like the sickle cell crises and are not associated with irreversibly sickled cells in the peripheral smear. Thus in this population, for the purpose of this screening study, any hemoglobin migrating to the position of S on the cellulose acetate membrane at pH 8.4 was considered conclusively as HbS.

Gene frequency of hemoglobin S: According to the 2001 census**,** the tribal population of Kerala totaled 3,64,189 which accounted for 1.14% of the total population. 37% of the scheduled tribes in Kerala reside in Wyanad while about 11.1% is residents of Attappady. Of the three tribal communities living in Attappady, Irula comprise 78% followed by Mudugas with 14% and the least of the lot being Kurumba with 8%. Literacy rates of all the three communities are in the 20s with Irula leading with 29.3% and Kurumba trailing with 22.3%.

All the three tribal communities in Attappady showed similar gene frequency for HbS. The highest frequency of 0.133 was found in both Irula and Muduga equally. This corresponds to a sickling positivity rate of 25%. This is slightly more than the sickling positivity rate of 21.8% that we got for Irula during our sickling test based pilot study conducted in 1989. Another sickling test based study conducted in 1960s[46] also showed a lower rate of 20.3%. It is interesting to note that the Irula community is one group who has advanced significantly up the socio-economic ladder over the last two decades. The literacy rate which was only 11.7% in 1981 has now climbed up to 29.3% in 1991 and possibly much more today overtaking all other tribal communities in Attappady. The high rate of female literacy of 25.7% and the fast rising numbers of Irula taking up tertiary occupations in the Government departments[17] are all pointers towards that. The survival of the homozygous SS cases is believed to have a direct bearing on the socio-economic status of the community[15]. This would have happened in the case of Irula thus increasing the gene frequency of HbS. Irula of Nilgiris of Tamil Nadu is reported to be having a much higher gene frequency of 0.177 for HbS[47].

Muduga are believed to be the earliest immigrants to Attappady region, thus giving them a head start for a higher gene frequency of HbS. In fact it can be argued that earlier the settlement in the area, more the chance for the gene frequency for HbS to build up in the population. This is explained by the survival advantage enjoyed by the carriers of the sickle cell gene as they have increased resistance to malaria[36]. And Attappady region is a known endemic area for malaria till a few decades back. Although they are considered superior to the Irula; Mudugas have become largely landless. The literacy rate, particularly the female literacy, is a dismal 17.1%. This has probably affected the survival of the diseased SS cases in their community pulling down the gene frequency. The total number of Mudugas tested in this study was only 45 and out of this one of the colony studied was in the Agali panchayat. Agali

panchayat showed a lower gene frequency compared to other panchayats as will be discussed later. This could be the other possible explanation for the lower than expected gene frequency in Muduga. No other data is available about the gene frequency of HbS in Muduga community.

Muduva, the community residing in Idukki, is confused with the Muduga of Attappady and even considered as one in the census reports. Interestingly Muduva are not known to have the sickle cell gene.

Kurumba of Attappady, also called as 'Palu Kurumba' to distinguish them from 'Alu Kurumba' of the Nilgiris, has a gene frequency of 0.108 corresponding to a sickling positivity rate of 21.5%. This is the lowest of all three communities in Attappady. Kurumba, fewest in number of the lot, were semi-nomadic in the past and still continue to abandon old sites for the new. They have a well established tradition of herbal medicine associated with magico-religious practices for curing most diseases. They also show a favourable attitude towards Modern Medicine and also towards family planning programmes. Kurumba are considered superior to even the Muduga though they inter-marry with them. No data is available about the gene frequency of 'Alu Kurmbas' living in the other side of the border in Nilgiris.

Kurumans of Wyanad are thought to be descendants of a ruling class of South India and related to the Kurumba of Nilgiris. Of them Thurston writes "The final overthrow of the Kurumba sovereignity was effected by the Chola king Adonal. The Kurumbas were scattered far and wide. Many fled to the hills in the Nilgiris and Wyanad, in Coorg and Mysore. Representatives of this ancient race are now found as wild and uncivilized tribes". According to their folklore, the Kurumas believe that they were the rulers of Wyanad in ancient times. They are socially and economically more advanced than all other tribal communities in Wyanad and are considered superior to all of them. The gene frequency of HbS among them is the highest among tribes of Wyanad at 0.180[15]. Their literacy rates are also among the highest among

tribes at 38.5%. The Kurumba of Attappady has not reached at that level with their literacy rate of 22.6%.

The major studies of the sickle cell gene from India are shown below in table 5-1, to compare with the situation in Attappady. Communities in whom there is a sickling positivity rate of more than 10% when a large enough sample (100 or more) is studied alone are shown. Where there is more than one such study in a community in a particular state, the one with the larger sample only is shown. The gene frequencies are all calculated by us from the electrophoresis data. The figures for gene frequencies are given only for studies in which hemoglobin electrophoresis has been done.

Table 5-1: HbS gene frequencies and Sickling rates in India

Community	N	Sickling %	Gene Frequency	Ref
Andhra Pradesh				
Koya	159	13.21	0.069	48
Rajgond	197	11.34	0.057	49
Pardhan	122	31.71	0.159	49
Kolam	215	14.88	0.077	47
Tamilnadu				
Soliga	115	26.02	--	46
Irula	130	35.39	0.177	47
Karnataka				
Yerava	131	23.66	--	46
Kerala				
Irula	184	20.27	--	46
Paniya	955	26.70	--	46
Madhya Pradesh				
Bhilala	384	31.51	0.176	50
Barela	345	25.51	0.132	50
Bade Bhatra	153	16.34	--	46
Muria	169	15.98	--	46
Dorla	200	13.00	--	46
Gond	284	17.96	0.090	50

Halba	122	13.90	0.070	50
Bhil	977	19.85	0.103	50
Patelia	166	22.29	0.120	50
Bison Horn Maria	442	18.55	--	46
Orissa				
Pareng Gadaba	225	12.44	--	51
Kondh	116	11.21	0.056	52
Maharashtra				
Pawara	219	17.80	0.089	53
Pardhan	146	33.50	0.168	53
Bhil	673	18.57	0.093	53
Gujarat				
Naik	174	16.09	0.080	54
Bhil	206	15.53	0.078	54
Gamit	207	31.40	0.157	54
Dhanka	215	20.47	0.102	54
Dudhara	115	26.09	--	55
Dubla	109	13.76	--	55
Dhodia	213	17.84	0.089	54
Rajasthan				
Bhil	276	13.40	--	46
Wyanad study				
Paniya	279	18.28	0.095	15
Kuruma	256	33.59	0.181	15
Adiya	91	31.87	0.181	15
Wyanadan Chetti	214	34.11	0.196	15
The present study				
Irula	590	25.0	0.133	
Kurumba	79	21.5	0.108	
Muduga	45	24.2	0.133	

The gene frequency of 0.133 seen in Irula and Muduga of Attappady is matched only by Pardhans of Andhra Pradesh and Maharashtra, Irula of Nilgiris in Tamil Nadu, Bhilala of Madhya Pradesh and Gamits of Gujarat apart from the three communities in Wyanad district which are the tribal communities of Paniya Kuruma and Adiya and the non tribal community the Wyanadan Chetti. The highest gene frequency recorded in India is in Wyanadan Chetti.

Hb pattern was analysed according to the sex of the subjects as shown in table 4-4. Although more than 25% of the males tested showed the presence of HbS, the proportion of homozygote SS cases were only 0.7%. This was in contrast to almost three times the rate of SS cases among the females tested (1.9%). While there can be no genetic explanation for this, particularly after a significantly large has been studied, the resilience of girl child to get over major illnesses in the childhood might give an edge over the male child. This however becomes relevant only in severely symptomatic disease which was not the case in sickle cell disease in Attappady as will be discussed below.

The mean age according to the sex and Hb pattern did not show any significant difference (Table 4-5). What is to be noted is that the mean age of the SS cases was also similar to the normal subjects studied. This clearly points to the fact that there is no significant mortality due to sickle cell anemia at a younger age. The great majority of the subjects in this survey were, however, below 30 years of age.

There seems to be a geographical variation in the incidence of the gene within Attappady region as shown in Tables 4-7 and 4-8. While the frequency is very similar in Pudur and Sholayur panchayats, the frequency in Agali is about a third less. This is not because of the difference in the proportions of various communities studied in each panchayats; as the variation is replicated exactly among the Irula community studied in the three panchayats also. Agali panchayat, where the frequency is less, is different from other areas in that it is much more 'urbanised' with the maximum number of settlers, government offices and financial enterprises. Whether this has led to a gene dilution of the tribal people living there is to be considered.

Disease characteristics: There were 10 cases of sickle cell anemia in our study. This is not large enough to make definitive statements about the disease characteristics. The detailed clinical work up of cases that is possible in a hospital setup was not possible in our study. This was because we did not know during the

clinical examination and blood collection in the field that we are dealing with a case of sickle cell anemia. On the other hand, it is a sample that is formed by an unbiased field study and as such valuable as an approximation to the condition prevailing in the population. In contrast, hospital based studies are likely to be biased in favour of severe cases with complications, which require admission. The details of the clinical and laboratory data are given in table 4-9.

Anemia was assessed clinically in all the subjects by looking for pallor of the conjunctiva, tongue and nails. Hemic murmurs were also looked for. Anemia, if present, was graded as mild or severe. Cases with gross pallor with or without hemic murmurs were graded as severe. We had to resort to this subjective method since we were using the finger prick method of blood collection and it was not possible to add Hb estimation also using the already minimum blood collected. Out of the 10 cases, only 4 were detected to have anemia clinically; out of which only one had gross pallor to be graded as severe.

The term sickle cell crises was introduced to describe recurring attacks of pain involving the skeleton, chest and abdomen[26]. We have used it in a broad sense to include all of the acute, symptomatic events that punctuate the course of sickle cell anemia. The characteristic bone and abdominal pains are intense enough to be remembered and usually medical help is sought for. Only one of our cases (10%) of sickle cell anemia gave a history of having had episodes of crises requiring hospital admissions. There is of course some variation in the tolerance threshold of pain that is sometimes culturally determined. Still, intense as the pain usually is, and bearing in mind that the average age of our SS cases is 17.3 years, this is significantly low figure. In a study of cases of mild sickle cell anemia in people over 30 years from Jamaica, it was noted that 13.3% of the patients never had symptoms of crises[35]. In another study of mild disease in Saudi Arabia, one third of the 54 adults had no symptoms[56]. Our figure of 90% not having any symptoms of crises is higher but are not strictly

comparable because the above quoted studies are all atleast partially clinic based and the mean age is also higher. The field based study conducted by us in Wyanad showed that 56.5% of cases did not have any symptoms that could be related to crises[15].

Leg ulcers are said to be a recurring problem in adult cases of sickle cell anemia. This occurs as a result of breakdown of the skin over the malleoli and distal portions of legs.[26] They are said to affect as many as 75% of patients living in tropical climates[57]. In a study of 270 mild cases of sickle cell anemia from Dhaharan, Saudi Arabia, no case of leg ulceration was found[56]. In our study also we did not encounter even a single case of leg ulcer at the time of survey. Neither did any of our cases give a history of having had a leg ulcer. Leg ulcers have a positive correlation with a low study state hemoglobin concentration and a low level of HbF and thus is an indicator of the severity of disease. The absence of leg ulcers in our study, which cannot be missed even in field based study, point to the generally milder nature of the disease in this population. The experience in Wyanad, where again the disease is of mild nature, was similar, with only one case out of the 23 SS cases giving a history of having had leg ulcer[15].

Splenomegaly was not present in any of our cases, though minimally palpable spleens could have been missed in the camp survey situation. However the absence of big splenomegaly may be of some significance as it may serve to exclude double heterozygote conditions like sickle cell -thallassemia.

The sickling syndromes are said to profoundly affect growth and development. In studies of the black population, although normal at birth, the heights and weights of children with sickle cell anemia are significantly delayed by 3 to 6 years. Thereafter the growth curves maintain a relatively normal configuration but deviate progressively from the normal curves, a pattern that is more prominent in boys than in girls[26]. While examining children in schools and hostels it is easy to spot children who are

considerably shorter than their peers. Striking short stature was not seen in any of our SS patients.

We have divided our cases of sickle cell anemia according to the severity of the disease into mild and severe. Mild cases are those that have not had more than one crisis episode and also not having severe pallor. One patient with no history of crises but having severe pallor was classified as severe. 80% of our cases were mild sickle cell anemia by these criteria and 20% severe.

Cerebrovascular accidents, bacterial meningitis and osteomyelitis, nephritic syndrome, femoral head necrosis, hepatic crisis, and retinopathy are among the serious complications of sickle cell anemia. Stroke occurs in 6 to 17% of black children and young adults with the disease[26,58]. The relative risk of bacterial meningitis among black patients is more than 300 compared to the general population[59]. Osteomyelitis especially by salmonella is another debilitating complication[60]. Though we have occasionally seen some of these complications in our hospital material, we did not encounter a single instance of these complications during our survey, and we feel that these complications are not very common. However, the number of cases is too small to make emphatic conclusions. The Saudi Arabian study quoted previously also found low levels of these complications with a larger sample and despite it including hospital cases[56].

Prognosis and Survival: It has already been mentioned that the clinical profile of our patients indicates a relatively mild form of the disease as compared to the disease in blacks. This is reflected in the survival figures also. The mean age of our patients is 17.3 years, which is only slightly lower than the mean of 18.4 years of the sample of normal subjects. Ages ranged from 12 to 26. Moreover 60% of our cases were totally asymptomatic.

The theoretical expectation of the number of cases of sickle cell anemia at birth in a population can be calculated from the gene frequency of HbA and HbS which in

turn can be calculated from the frequency of AA and AS patterns in hemoglobin electrophoresis. Any deficit in the number of cases actually found in the survey, as compared to the expected number is an indicator of mortality. This data for different communities covered in our survey can be seen in table 4-10. We calculated the expected number of SS homozygotes in large electrophoresis based studies reported from different states for comparison. This is shown in table below.

Table 5-2: Expected and Actual SS Cases in different studies

Study (State)	N	SS Cases		Ref
		Expected	Actual	
Blake et al (Andhra Pradesh)	586	5.0	3	48
Goud & Rao (Andhra Pradesh)	1110	4.6	0	49
Saha et al (Tamilnadu)	316	4.1	2	61
Banker et al (Maharashtra)	1962	12.4	0	53
Vyas et al (Gujarat)	1408	12.0	0	54
IIH, ICMR (Madhya Pradesh)	2886	33.7	21	50
Feroze et al (Wyanad, Kerala)	973	21.8	23	15
This study (Attappady, Kerala)	714	12	10	--

Thus, while all other studies in India show a marked deficit of cases, this is not seen in our material. Even giving some allowance for some bias towards case detection, this is remarkable. Further, such biases, if any, may be operating in the other studies also. It would be too far fetched to think of sickle cell anemia in Kerala to be genetically different from that in the rest of India. We contend that the disease is generally of a milder nature in India as a whole when compared to Africa and America. Access to primary health care even without sophisticated technology and drugs would ensure fairly good survival. Access to the modern health care system to the same extent as in Kerala is lacking in the other states and the problem is more acute in case of tribals and other disadvantaged groups.

Socio-economic advancement and entitlements may have a critical role to play in the increase in survival of sickle cell anemia patients. The present study and the study in Wyanad show the variations in the level of deficit between communities to be broadly related to the literacy rates of the communities, which give an idea about the socio-economic status. The increase in the gene frequency of HbS in Irula over the years, as evidenced by the three studies conducted among them spread over the last 40 years may well be due to increased survival of their SS cases. And there is evidence to suggest the leap forward the community has taken in the socio-economic front over the last two to three decades.

In the United States and Caribbean, a much more severe form of the disease is contained and survival into middle age assured for an equal or even more proportion of cases. This is not by any newer technological wonders but by simple case detection, health education and simple protocol based management. There is no reason why the same strategies cannot be applied to Kerala with even better success given the milder nature of the illness.

Hypochromia of the red cells in the peripheral smear was looked into in every sample. Hypochromia microcytic anemia can be taken as an indicator of iron deficiency in the individual, which is the commonest nutritional deficiency in a given population of third world countries. So frequency of hypochromia roughly points to the nutritional status of the community. The other important condition that can produce hypochromia in the red cells is thalassaemia. But the possibility of this population having beta-thalassaemia is highly improbable because we did not find any single case of homozygous beta thalassaemia in our material. Splenomegaly, which is usually seen in sickle cell - thalassaemia was also not observed in any of our patients. Prominent HbA_2 band in Hb electrophoresis seen in beta thalassaemia was totally missing from the samples we tested.

A total of 90 cases of hypochromic microcytic anemia were picked up from the samples studied, of which 19 were severe and the rest moderate. This comprised 12.6% of the tribals tested (Table 4-11). Interestingly, 25 of them were detected from one centre which is the Tribal Hostel in Pudur. That clearly indicates some imbalance in the nutrition that is being catered in the hostel. It also underscores the fact that the hypochromia seen in our cases are due to nutritional deficiency and not thalassaemia. If those cases are omitted, the overall iron deficiency among the tribal communities would be only 10.4%. This clearly negates the general impression that the tribal communities have widespread nutritional deficiency due to starvation. It could also mean that whatever food they are taking are well balanced for the various minerals. Community wise break-up showed the lowest frequency among Muduga and highest among Kurumba.

We have made some projections of the number of cases of sickle cell anemia in Attappady region as also the number of infants who will be born with the disease each year. The projections for the 2001 population of various communities are made based on the 1991 census report as the base year and the growth rates between 1981 and 1991. The expected number of SS cases is worked out on the basis of the gene frequencies of the HbS gene and not by extrapolation from the percentage of SS cases obtained in the survey.

Table 5-3: Projection of Sickle cell anemia (SS) cases in Attappady

Community	Projected pop.(2001)	Expected SS cases
Irula	25,502	451
Kurumba	2581	30
Muduga	4740	84
Total	**32,823**	**565**

So the total size of the problem in Attappady is about 550 to 600 SS cases that need individualized attention. An additional 10 to 15 newborns with homozygous state is expected every year. The heterozygotes, numbering to slightly more than 8000, need to be diagnosed and properly recorded as part of the long term management and control of the disease. And with the milder nature of the disease it should not be a problem to give the disadvantaged a decent quality of life if the Government and the medical fraternity are sincerely committed to it.

CONCLUSIONS
AND
RECOMMENDATIONS

The problem of hemoglobinopathy in Attappady region as revealed in this study can be summarized as follows.

1. Sickle cell gene shows high prevalence among all the three tribal communities living in the region. The community and the gene frequency are
    a. Irula – 0.133
    b. Kurumba – 0.108
    c. Muduga – 0.133
2. Other hemoglobinopathies or thalassaemias are not prevalent in this population to any significant extent.
3. Sickle cell anemia in Attappady is a relatively mild disease compared to that seen in Africa or African immigrants in US. It can be compared to that seen in Wyanad.
4. There is no deficit of SS cases in this study in contrast to population screening studies from other states of India. What this implies is that the survival of sickle cell anemia patients is more in Kerala when compared to the rest of India. As there is no reason to believe that the genetic origins of HbS is different in Kerala and the rest of India, it would seem that even small improvements in Primary Health Care made available to the population (as in Kerala) is sufficient to achieve this effect.

5. The estimated number of sickle cell homozygotes in Attappady is 565 and the immediate challenge would be to put in place a system to identify them and give individual attention to them.

What is to be done: The two strategical options in genetic disorders are attempts at decreasing gene frequency and disease management. In the case of sickle cell anemia in Attappady the first option is not feasible given the small community sizes and general backwardness of the population concerned. While genetic counseling should be made available on demand, it has no place in a public health campaign. Moreover, since the disease in this area is generally mild, the emphasis should definitely be on disease management.

Disease management: A scheme for steady state management of sickle cell anemia, followed in the United States and countries like Saudi Arabia where there is a good proportion of severe form of disease is given below. With suitable modifications to tackle a mild form of disease, it can be taken as broad guidelines.

Steady state management (children):

Birth – cord blood electrophoresis

4-6 months – routine hematology
repeat electrophoresis
regular checkup (2-4 months)
folid acid supplementation
start penicillin prophylaxis
screen parents
genetic counseling
patient and family education

1 year – H influenza vaccination (and continue every 2 years)
regular checkup
health counseling

2 years – start pneumococcal vaccination (and continue every 2 years)

5-6 years – abdominal ultrasound (baseline)

9-10 years – abdominal ultrasound (and later every 6 months)

eye examination by ophthalmologist – yearly

health counseling

Steady state management (adult):

- prevention of further complications
- early recognition and treatment of complications
- continued parent education
- regular checkup (4-6 months)
- complete blood count and retic count
- routine chemistry
- organ function tests
- urinalysis

Laboratory diagnosis:

Ideally the following laboratory investigations should be available at some level in the health delivery system.

I) Obligatory tests

(a) RBC morphology – from blood smear

(b) Routine blood tests (Hb; RBC, WBC and Platelet counts; hematocrit; RBC indices; retculocyte count)

(c) Sickling/solubility tests

(d) Hemoglobin electrophoresis at alkaline/acidic pH

(e) Measurement of hemoglobins A_2 and F

(f) Serum iron, TIBC, serum ferritin

II) Special tests

(a) Family studies

(b) Measurement of alpha/non alpha globin chain ratios

(c) Structural studies (Hb fingerprinting)

(d) Gene studies (restriction endonuclease digestion, PCR, Allele specific oligonucleotide hybridization etc)

As can be seen from above, the programme is an exhaustive one and targeted to severe forms of sickle cell anemia. The important point to be adopted is putting a system in place for identifying and targeting sickle cell anemia cases. Having a milder form of disease in hand, focus should mainly be on providing immediate attention to potential factors that can precipitate sickle cell crises.

In this context it is important to discuss the role of vaccinations in sickle cell anemia. There is no doubt that vaccinations do reduce the occurrence of diseases for which it is given. But seeing that in isolation and giving vaccinations indiscriminately to even heterozygotes and normal subjects can only be seen as gross misappropriation of the preciously scarce resources. The primary objective should be to put in place a system to make proper diagnosis of all the patients and then give access to primary care to them in a priority basis. The incredible change this cost effective system can bring to the survival and quality of life of sickle cell anemia patients can easily be comprehended by noticing the reduction in deficit of SS cases in Wyanad and Attappady compared to the rest of India. Small planned increments to the existing system can raise the life expectancy of the SS cases to almost that of normal population.

We feel that the format of diagnosis and management of sickle cell anemia ideal to our situations can be gradually introduced and incorporated into the health care delivery system at three levels. The functions of each level could be as follows.

Comprehensive health care system:

Management at three levels

Level I - Primary health center:

- Provisional diagnosis using screening tests like sickling / solubility tests
- Patient care and follow-up
- Maintenance of patient records
- Health education and counseling to parents
- Communication with levels II and III

Level II - Taluk hospitals / Community health center:

- Definitive diagnosis using Hb electrophoresis and other obligatory tests
- Patient care and follow-up
- Management of acute emergencies
- Maintenance of regional records
- Health education and counseling to parents
- Social support
- Training for health personnel
- Communication with levels II and III

Level III - Medical college, Calicut:

- Definitive diagnosis including special tests to detect combinations with other hemolytic anaemias and DNA diagnostics
- Specialised treatment for complications
- Management of acute emergencies
- Management consultation
- Maintenance of state registry of patients
- Provision of information on sickle cell anemia to general public through lectures, pamphlets, videotapes

- Organizing state and national symposia
- Training workshops for health personnel including doctors
- Communication and support back up

The problem of sickle cell anemia in Attappady and Wayanad is going to be an expanding one because more and more homozygotes are going to survive and reach adulthood. The scheme outlined above can be implemented in stages and integrated into the health services by the government if it is earnest in tackling the problem. For this to be effective, certain infrastructural changes and human resource development measures have to be undertaken urgently.

Doctors and health workers can be trained in batches using the facilities of the Medical College at Calicut. Two day long, module based workshops can be conducted separately for doctors and health workers incorporating the following.

(a) Different clinical manifestations

(b) Genetics of hemoglobinopathies and scope of counseling

(c) Diagnostic methods

(d) Simple protocol based management

The following infrastructural needs may also be given priority

(a) Provision of diagnosis by sickling test and blood smear in all the primary health centres

(b) Hemoglobin electrophoresis test in the taluk hospital and community health center

(c) Facilities for blood transfusion at the taluk hospital and later at Community health center, Agali and Pudur primary health center.

(d) Sickle cell clinics at Agali, Pudur and Sholayur health centers.

(e) Establishment of a Hemolytic anemia reference and coordinating centre at the Medical College, Calicut

Finally, we would like to caution against the tendency to see sickle cell anemia as a problem in isolation crying out for a pure technical solution. We believe, the most important message of this study is that, better survival and quality of life of the affected people are dependent on the primary health care that they receive, which in turn is dependent on their overall socioeconomic condition. It is our fond hope that whatever technical measures that are contemplated be within a framework of efforts for overall empowerment of these communities.

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