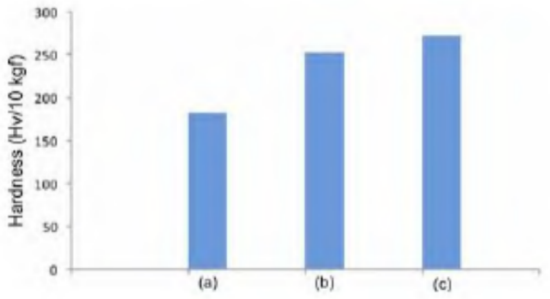

Figure 5. Variation in hardness of P/M alloys (a), (b) and (c).

Table 2. Tensile properties of P/M alloys in fully annealed condition

Sample	Proof stress (MPa)	UTS (MPa)	Total elongation (%)
a	171	296	13
b	282	663	12
c	290	675	14

2Cu–1Si–0.5Mo having 1 vol% porosity and Fe–0.35P–2Ni–2Cu–1Si–0.5Mo–0.15C with 1.03 vol% porosity showed considerable improvement in hardness. Their hardness values are 253 and 272 Hv/10 kg respectively. Had there been similar porosity levels in both of these alloys, improvement in hardness due to phosphorus, molybdenum and nickel alloying addition could have been ascertained.

All the Fe–P alloys showed similar level of ductility. However, the Fe–P based alloys containing other alloying elements (Mo, Ni, Si, C and Cu) show fairly high strength as compared to the one containing 0.35 wt% P. Tensile properties, such as proof strength, tensile strength, percentage elongation of these alloys are shown in Table 2. The mechanical properties obtained in the present investigation are suitable for structural applications. Few limited tensile tests under cold deformed conditions exhibited UTS well over 900 MPa. However ductility came lower marginally. This is possible on account of development of finer grain grain structures due to cold working.

The following conclusions can be drawn from the present investigation are: (i) alloys developed in the present investigation have very good hot workability. (ii) High strength and high ductility are observed in case of alloys containing 0.35 wt% phosphorous. (iii) Alloys containing Mo, Ni, Cu and Si (with or without carbon) showed higher strength (>600 MPa) and higher resilience value with moderate ductility under annealed conditions with scope for developing higher strengths by cold working. (iv) Forged and homogenized as well as rolled and annealed Fe–P based alloys developed in the present investigation were characterized using metallographic technique. All the microstructures showed single-phase ferrite grains with porosities well distributed along the grain boundaries as well as inside the grains. (v) Improvement in hardness levels due to the combined addition of molybdenum, nickel silicon, copper, carbon and phosphorus was found to be comparatively better than that of binary alloys. (vi) Copper significantly reduces the porosity. Binary alloy (Fe–0.35P) shows higher levels of porosity than other two alloys developed in the present study.

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Phenotypic diversity of sickle cell disorders with special emphasis on public health genetics in India

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Human genetic diversity poses a great challenge to community health care in India. Haemoglobin disorders constitute the most common genetic and public health burden on vulnerable people. Prospective studies which are lacking in India present valuable community health and morbidity information for analysis with respect to introspection and evaluation. The present study is designed to fill up this lacuna in presenting community health and morbidity pattern of encountered different sickle cell phenotypes in India. 137

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suspected cases of sickle cell disorders referred to our centre for laboratory investigations were analysed using an automated blood cell counter, alkaline electrophoresis and haemoglobin variant analyser. From a community perspective, transfusion dependent sickle cell disease and sickle cell-β-thalassemia were found prevalent in India. Statistically significant differences in haematological indices were observed between sickle cell trait and sickle cell disease, sickle cell-β^{++}-thalassemia, and sickle cell-β^{+}-thalassemia; between sickle cell disease and sickle cell-β^{++}-thalassemia and sickle cell-β^{+}-thalassemia; and between sickle cell-β^{++} thalassemia and sickle cell-β^{+}-thalassemia with variable symptomatology and clinical manifestations. Genetic heterogeneity of sickle cell-β-thalassemia was noticed in India. Both mild (African) and severe (Mediterranean) forms of sickle cell-β-thalassemia (i.e. β^{++}-thalassemia and β^{+}-thalassemia or β°-thalassemia) were encountered suggesting gene flow from the East Mediterranean, Asian Gulf, sub-Sahara and East Africa, reflecting historical events and gene migrations in the region having implications in community health and public health genetics in India.

Keywords: Genetic diversity, sickle cell phenotypes, sickle cell trait, sickle cell-β^{++}-thalassemia, sickle cell-β^{+}-thalassemia.

IN view of the vast social stratification, ecology and genetic diversity, the community health genetics all over the world including India has got a major medical thrust for nascent preventable and emerging health problems in terms of diagnosis, clinical management and genetic counselling. Because of potential predictable benefits, recent technological advancements have revolutionized the scientific tempo in population health, medical and molecular genetics and brought about solutions to many preventable genetically inherited chronic diseases. Congenital haemoglobin (Hb) disorders constitute one of the such public health and genetic maladies.

Haemoglobinopathies are the commonest, life-threatening, monogenic disorders in the world. Fairly recent estimates suggest that 7% of the world populations are carriers and that 300,000–400,000 affected children are born every year[1]. A majority of these have sickle cell disease (~250,000). The highest frequency of sickle cell disease is in tropical regions, particularly sub-Saharan Africa, India and the Middle East[1]. Sickle cell haemoglobin (Hb S) is one of the major haemoglobin disorders, formed as a result of a single-gene defect causing substitution of valine for glutamic acid in 6th position of the β-globin chain of the haemoglobin molecule. Persons homozygous for Hb S have sickle cell anaemia. Under conditions of low oxygen tension, sickle haemoglobin polymerizes causing the red blood cells to assume a sickled, crescent or banana shape in persons with sickle cell anaemia[2]. This deformity of red blood cells leads to the symptoms of sickle cell disease.

Beta (β)-thalassemia syndromes are a group of hereditary disorders characterized by a genetic deficiency in the synthesis of β-globin chains. In the homozygous state, β-thalassemia (i.e. thalassemia major) causes severe transfusion-dependent anaemia. In the heterozygous state, the β-thalassemia trait (i.e. thalassemia minor) causes mild-to-moderate microcytic anaemia. β-thalassemia is caused by a genetic mutation in the β-globin gene; however, many additional factors influence the clinical manifestations of the disease. That is, the same particular mutation may have different clinical manifestations in different patients. The following factors are known to influence the clinical phenotype in Hb disorders: (i) Intra-cellular foetal haemoglobin (Hb F) concentrations: (a) Level of expression of Hb F (i.e. the expression of the β-globin gene) determines, in part, the severity of disease. (b) Patients with high Hb have milder disease. (ii) Co-inheritance of α-thalassemia: (a) Patients with co-inheritance of α-thalassemia have a milder clinical course because they have a less severe α–β chain imbalance. (b) The coexistence of sickle cell trait and β-thalassemia is a major symptomatic haemoglobin disorder with most of the symptoms and complications of the sickle cell disease. Persons heterozygous for sickle Hb and β-thalassemia (Hb S/β-thal) also may experience sickle cell disease, although their symptoms tend to be less severe than those persons homozygous for sickle cell disease.

The sickle cell-β-thalassemia varies in severity, depending on the inherited β-thalassemia mutation. There are two main varieties of β-thalassemia in different populations, β°-thalassemia, in which no β-globin is produced, and β^{+}-thalassemia, in which some β-globin is produced, but less than normal. The diagnostic feature of β-thalassemia is an elevated level of Hb A_2 in heterozygotes, which is found in most forms of β° and β^{+} thalassemia. Comparatively, less severe form of β-thalassemia is sometimes designated β^{++}-thalassemia to indicate that the defect in β-chain production is particularly mild with relatively high (20–30%) levels of Hb A. The latter may be further divided into β^{+} and β^{++}-thalassemia, reflecting variability in the degree of severity of the defect in β-globin production[3]. Sickle cell-β^{+}-thalassemia tends to be less severe than sickle cell-β°-thalassemia. Patients with sickle cell-β°-thalassemia tend to have more irreversibly sickled cells, more frequent vaso-occlusive problems, and more severe anaemia than those with sickle cell-β^{+}-thalassemia. It is often difficult to distinguish between sickle cell disease and sickle cell-β°-thalassemia. There are, however, less common forms of β-thalassemia in which the Hb A_2 level is normal in heterozygotes. Broadly, they are classified into varieties, depending on the clinical manifestations and levels of Hb F and A_2 in heterozygotes: type 1 (silent β-thalassemia), in which there are no associated haematological changes, and type 2, in which the haematological findings are typical of β-thalassemia trait with a raised Hb A_2 level. Both these

varieties are heterogeneous at the molecular level[3]. The level of adult Hb (Hb A) in patients with sickle cell-thalassemia depends on a particular β-thalassemia mutation. The Hb S/β^{++} thalassemia substitution (–88 C→T) with Hb A in the range of 20% (African), Hb S/β^{+}-thalassemia (IVS1-5 G→A) with Hb A in the range of 10% (Mediterranean or Middle East) and Hb S/β°-thalassemia (IVS1-1 G→A) without Hb A are common in India[3–5].

Both sickle cell disease (1–44%) and β-thalassemia syndromes (3–17%) are prevalent in India[4,6–8]. There exists a sickle cell belt in central India covering the states of Jharkhand, Orissa, Chhattisgarh, Andhra Pradesh, Tamil Nadu, Kerala, Maharashtra, Gujarat, Rajasthan, Madhya Pradesh and eastern Uttar Pradesh. Similarly, except southern Indian states, β-thalassemia is encountered all over India. To the best of our knowledge, there is no exclusive report on different prevalent phenotypes of the sickle cell disease in India. This clinical study highlights the phenotypic diversity of encountered sickle haemoglobin disorders in haematological profile with special reference to the state of Orissa in central-east coast of India.

This study is based on suspected routinely referred cases of haemoglobinopathies to our Centre with anaemia/body pains for detailed investigations from different peripheral primary health centres (PHCs) and hospitals in Orissa. All such cases with their family members such as parents, brother/sister were also subjected to clinical examination and laboratory investigation after taking informed consent for genetic/marriage counselling. In addition to recording of clinical signs and symptoms, background information for each subject such as name, age, sex, caste, native place, reproductive history, family pedigree was also noted.

About 2–3 ml intravenous blood samples were collected using ethylene diamine tetra acetic acid (EDTA) as anticoagulant by disposable syringes and needles from each individual under aseptic conditions. All the signs and symptoms related to Hb S/β-thalassemia syndromes were recorded on the pre-designed proforma after clinical examination. Laboratory biochemical investigations were carried out following the standard procedures after cross checking for quality control from time to time. Haematological parameters such as Hb level, red blood corpuscle (RBC), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell width (RDW), white blood corpuscle (WBC) using an automated Blood Cell Counter (model: MS4, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France), and Hb A_2 level, Hb F, Hb S and Hb A were studied by carrying out biochemical analysis.

The sickling test was performed by using freshly prepared sodium metabisulphite solution as reducing agent for the presence or absence of Hb S[9]. The routine Hb lysate electrophoresis was carried out on cellulose acetate membrane (CAM) in tris-EDTA-borate buffer at pH 8.9 and quantification of Hb A_2 fraction was done by elution method[10]. Value more than 3.5% of Hb A_2 fraction of Hb A was taken as cut-off point for determining the β-thalassemia trait. Estimation of Hb F was done as described by Weatherall[10].

The sickle cell-β-thalassemia diagnosis was based on the findings of A, F, S and A_2 on electrophoresis under alkaline media, elevated Hb A_2 levels (> 3.5%) and family study. In view of the inverse relationship between Hb A_2 and Hb F levels, the high levels of Hb F were common in Indian sickle cell patients[11–13]. All the blood samples were further subjected to confirmation by Hb variant analysis (made for Bio-Rad Diagnostics, Hercules California, USA).

There were in all 137 cases, out of which 64 (31 males and 33 females) were sickle cell trait, 29 cases (16 males and 13 females) sickle cell disease, 14 (6 males and 8 females) sickle cell β^{++}-thalassemia and 30 (20 males and 10 females) sickle cell-β^{+}-thalassemia included in the present comparative study. Results were statistically analysed and tested using student's *t*-test for significance, if any, between the different diagnostic groups.

It is apparent from Table 1 that the sex differences for mean values of the studied hematological indices between different diagnostic categories of sickle cell phenotypes are statistically not significant except in the sickle cell carriers. The red cell indices like mean Hb level ($P < 0.0005$), red cell counts ($P < 0.04$) and haematocrit ($P < 0.002$) was statistically lower in females than in the males of sickle cell trait. Sex differences in age for sickle cell traits were also observed. Mean Hb A_2 level in diagnostic category of sickle cell-β^{++} thalassemia was also statistically higher in females than in males ($P < 0.001$) in the present study.

Since there were no statistically significant sex differences observed for different haematological indices in the studied diagnostic categories, therefore, their mean values were pooled together for comparison purposes (Table 2). It was interesting to note that the red cell indices of pooled sickle cell carriers showed statistically highly significant higher mean values in Hb level ($P < 0.001$), RBC count ($P < 0.001$), HCT ($P < 0.001$) and Hb A_2 level ($P < 0.05$) than in the sickle cell disease patients except MCV, MCH and MCHC (Table 2). Statistically significant higher mean values were observed in sickle cell disease patients than in the sickle cell traits for RDW ($P < 0.03$), WBC count ($P < 0.001$), Hb F ($P < 0.001$), and the percentage of Hb S ($P < 0.001$).

In comparison to sickle cell trait, the mean values of red cell indices (sexes combined) were statistically significantly much lower for Hb level ($P < 0.001$), RBC count ($P < 0.01$), HCT ($P < 0.001$), MCV ($P < 0.001$), MCH ($P < 0.001$), MCHC ($P < 0.001$) and Hb A in sickle cell-β^{++}-thalassemia cases (Hb A > 10%). However, some indices like RDW ($P < 0.001$), WBC ($P < 0.001$),

Table 1. Mean values of haematological indices in both sexes in different sickle cell phenotypes in India

Haematological parameters	Sickle cell trait		Sickle cell disease		Sickle cell-β^{++}-thalassemia Hb A > 10%		Sickle cell-β^{+}-thalassemia Hb A < 10%	
	n = 31 (M)	n = 33 (F)	n = 16 (M)	n = 13 (F)	n = 6 (M)	n = 8 (F)	n = 20 (M)	n = 10 (F)
Age in years	35.5 ± 16.2[a]	28.0 ± 11.6	08.8 ± 07.5	13.0 ± 08.5	11.5 ± 08.8	12.2 ± 10.2	14.5 ± 00.7[f]	03.0 ± 00.7
Hb (g/dl)	12.7 ± 01.5[b]	11.4 ± 01.3	07.4 ± 01.6	07.8 ± 01.7	08.4 ± 02.6	08.2 ± 02.6	08.7 ± 03.1	07.7 ± 00.7
RBC ($\times 10^6$/µl)	05.0 ± 00.9[c]	04.6 ± 00.7	02.8 ± 00.8	03.0 ± 01.0	04.4 ± 00.9	04.0 ± 01.2	04.5 ± 01.1	03.7 ± 03.2
HCT (%)	40.4 ± 05.1[d]	36.6 ± 04.7	23. ± 05.4	25.5 ± 06.7	31.7 ± 06.7	31.4 ± 09.7	30.0 ± 08.5	28.0 ± 01.2
MCV (fl)	80.8 ± 09.0	80.7 ± 08.4	82.9 ± 11.3	81.0 ± 14.9	70.6 ± 03.7	76.3 ± 11.5	65.8 ± 02.8	63.9 ± 03.1
MCH (pg)	25.4 ± 03.5	24.8 ± 03.8	26.5 ± 04.5	26.4 ± 05.4	19.8 ± 03.8	21.9 ± 04.6	18.8 ± 02.3	20.6 ± 02.1
MCHC (g/dl)	31.2 ± 02.4	30.8 ± 02.0	30.8 ± 04.3	31.2 ± 04.1	26.9 ± 03.6	27.8 ± 03.2	28.6 ± 02.0	29.6 ± 00.2
RDW (%)	08.7 ± 00.6	08.8 ± 01.1	15.0 ± 06.8	11.2 ± 02.4	11.1 ± 03.0	10.7 ± 02.4	11.4 ± 01.3	08.6 ± 00.3
WBC ($\times 10^3$/µl)	08.0 ± 01.5	08.6 ± 02.0	15.3 ± 08.5	13.5 ± 07.9	13.3 ± 05.0	15.1 ± 11.1	12.6 ± 07.0	17.9 ± 01.4
Hb A_2 (%)	02.3 ± 00.5	02.1 ± 00.4	01.9 ± 00.5	02.0 ± 00.6	04.0 ± 00.3[e]	04.9 ± 00.9	06.4 ± 02.3	03.9 ± 00.2
Hb F (%)	01.0 ± 01.3	01.1 ± 00.8	14.7 ± 07.5	18.2 ± 06.0	10.6 ± 06.7	12.4 ± 12.8	15.7 ± 05.7[f]	38.1 ± 01.3
Hb S (%)	27.4 ± 06.8	29.0 ± 09.6	83.4 ± 07.4	79.8 ± 06.2	50.0 ± 19.0	55.7 ± 15.8	71.2 ± 03.1[f]	51.7 ± 04.3
Hb A (%)	69.5 ± 07.2	67.7 ± 09.6	–	–	33.9 ± 23.3	27.0 ± 21.0	06.8 ± 00.3	05.5 ± 01.5

Sex difference significant: [a]$P < 0.03$; [b]$P < 0.0005$; [c]$P < 0.04$; [d]$P < 0.002$; [e]$P < 0.001$; [f]$P < 0.01$.

Table 2. Mean values of haematological indices (sexes combined) of different sickle cell phenotypes in India

Haematological parameters	Sickle cell trait n = 64 1	Sickle cell disease n = 29 2	Sickle cell-β^{++}-thalassemia Hb A > 10% n = 14 3	Sickle cell-β^{+}-thalassemia Hb A < 10% n = 30 4	Significant between 1, 2 $P <$	1, 3 $P <$	1, 4 $P <$	2, 3 $P <$	3, 4 $P <$
Age in years	28.0 ± 11.6	13.0 ± 08.5	12.2 ± 10.2	10.7 ± 06.7	0.001	0.001	0.01	–	–
Hb (g/dl)	11.4 ± 01.3	07.8 ± 01.7	08.1 ± 02.6	08.4 ± 02.3	0.001	0.001	0.002	–	–
RBC ($\times 10^6$/µl)	04.6 ± 00.7	03.0 ± 01.0	04.0 ± 01.2	04.2 ± 00.9	0.001	0.01	–	0.001	0.01
HCT (%)	37.0 ± 04.7	26.0 ± 06.7	31.0 ± 09.7	29.3 ± 06.1	0.001	0.001	0.01	0.01	–
MCV (fl)	80.8 ± 08.4	81.0 ± 14.9	76.3 ± 11.4	65.2 ± 02.3	–	0.001	–	0.03	0.03
MCH (pg)	24.8 ± 03.8	26.4 ± 05.4	22.0 ± 04.6	19.4 ± 01.9	–	0.001	0.01	0.01	0.02
MCHC (g/dl)	30.8 ± 02.0	31.2 ± 04.0	27.8 ± 03.2	29.0 ± 01.6	–	0.001	–	–	–
RDW (%)	08.8 ± 01.1	11.2 ± 02.4	10.7 ± 02.4	10.5 ± 01.8	0.03	0.001	0.01	–	–
WBC ($\times 10^3$/µl)	08.6 ± 02.0	13.5 ± 07.9	15.1 ± 11.1	14.3 ± 05.8	0.001	0.001	–	0.001	–
Hb A_2 (%)	02.1 ± 00.4	02.0 ± 00.6	04.9 ± 00.9	05.6 ± 02.1	0.05	0.001	0.001	–	0.001
Hb F (%)	01.1 ± 00.8	18.2 ± 06.0	12.4 ± 12.8	23.1 ± 13.6	0.001	0.001	0.001	0.001	–
Hb S (%)	29.0 ± 09.6	79.8 ± 06.2	55.7 ± 15.8	64.7 ± 11.5	0.001	0.001	0.001	–	0.001
Hb A (%)	67.7 ± 09.6	–	27.0 ± 21.0	06.3 ± 00.8	–	–	0.001	–	–

Hb A_2 ($P < 0.001$), Hb F ($P < 0.001$) and Hb S ($P < 0.001$) showed statistically significantly higher mean values in sickle cell-β^{++}-thalassemia cases than in the sickle cell carriers (Table 2).

There was a further statistically significant reduction in the mean values of red cell indices like Hb level ($P < 0.002$), RBC count, HCT ($P < 0.01$), MCV, MCH ($P < 0.01$), MCHC and Hb A ($P < 0.001$) in sickle cell-β^{+}-thalassemia (Hb A < 10%) cases in comparison to sickle cell carriers (Table 2). However, the red cell indices such as RDW ($P < 0.01$), WBC count, Hb A_2 ($P < 0.001$), Hb F ($P < 0.001$) and Hb S level ($P < 0.001$) showed statistically significant higher mean values in sickle cell-β^{+}-thalassemia cases than in the sickle cell traits (Table 2).

There was statistically highly significant difference between the homozygous sickle cell disease and sickle cell-β^{++}-thalassemia cases (Table 2) with respect to RBC counts ($P < 0.001$), HCT ($P < 0.01$), MCV ($P < 0.03$), MCH ($P < 0.01$), WBC counts ($P < 0.001$) and Hb F ($P < 0.001$).

Statistically significant differences (Table 2) were also observed between the sickle cell-β^{++}-thalassemia (Hb A > 10%) and sickle cell-β^{+}-thalassemia (Hb A < 10%) cases for haematological parameters such as RBC counts ($P < 0.01$), MCV ($P < 0.03$), MCH ($P < 0.02$), Hb A_2 ($P < 0.001$) and the percentage of Hb S ($P < 0.001$).

Taking the clinical profile into consideration, the distribution of clinical symptoms such as pallor, fatigue and loss of appetite can be graded into sickle cell carriers (1.6%) < sickle cell-β^{++}-thalassemia (28.6%) < sickle cell-β^{+}-thalassemia (30.0%) < sickle cell disease (31.0%) cases (Table 3). Recurrent fever with cold and cough infections is common in sickle cell disease (44.8%),

Table 3. Clinical findings in different sickle cell phenotypes (sexes combined) in India

Clinical features	Sickle cell trait $n = 64$		Sickle cell disease $n = 29$		Sickle cell-β^{++}-thalassemia Hb A > 10% $n = 14$		Sickle cell-β^{+}-thalassemia Hb A < 10% $n = 30$	
	n	%	*n*	%	*n*	%	*n*	%
Pallor	1	1.6	9	31.0	4	28.6	9	30.0
Fatigue (weakness)	0	0.0	4	13.8	0	0.0	4	13.3
Loss of appetite	0	0.0	4	13.8	0	0.0	3	10.0
Dysponea	0	0.0	2	6.9	0	0.0	1	3.3
Recurrent fever with cold and cough	1	1.6	13	44.8	2	14.3	7	23.3
Recurrent jaundice	0	0.0	13	44.8	1	7.1	4	13.3
Dactylitis (hand and foot syndrome)	0	0.0	3	10.3	0	0.0	0	0.0
Typical haemolytic face	0	0.0	5	17.2	0	0.0	3	10.0
Abdominal pains	1	1.6	4	13.8	1	7.1	3	10.0
Chest pains	0	0.0	1	3.4	0	0.0	1	3.3
Bone joint pains	0	0.0	12	41.4	2	14.3	5	16.7
Epistaxis (nose bleeding)	0	0.0	1	3.4	0	0.0	1	3.3
Splenomegaly	1	1.6	3	10.3	1	7.1	3	10.0
Haepatomegaly	0	0.0	2	6.9	0	0.0	1	3.3
Aplastic crisis	0	0.0	2	6.9	0	0.0	0	0.0
Splenic sequestration	0	0.0	3	10.3	0	0.0	0	0.0
Cardiac murmurs	0	0.0	1	3.4	0	0.0	2	6.7
Loss of weight	1	1.6	5	17.2	0	0.0	3	10.0
Retardation of growth and development	1	1.6	3	10.3	1	7.1	3	10.0
Delayed menarche	0	0.0	3	10.3	0	0.0	3	10.0
History of blood transfusion	0	0.0	10	34.5	1	7.1	3	10.0
History of hospitalization	0	0.0	3	10.3	1	7.1	3	10.0

followed by sickle cell-β^{+}-thalassemia (23.3%) and sickle cell-β^{++}-thalassemia cases (14.3%). However, the occurrence of recurrent jaundice was the highest in sickle cell disease (44.8%), followed by sickle cell-β^{+}-thalassemia (13.3%) and sickle cell-β^{++}-thalassemia (7.1%) cases. The hand and foot syndrome (dactylitis) was prevalent only in sickle cell disease (10.3%) patients in India.

The typical haemolytic face was observed in sickle cell disease (17.2%) and sickle cell-β^{+}-thalassemia (10.0%) patients only. The distribution of abdominal pains can be graded into sickle cell carriers (1.6%) < sickle cell-β^{++}-thalassemia (7.1%) < sickle cell-β^{+}-thalassemia (10.0%) < sickle cell disease (13.8%) cases (Table 3). Chest pains were common only in sickle cell disease (3.4%) and sickle cell-β^{+}-thalassemia (3.3%) patients. Bone joint pains were common in all patients except the sickle cell traits. Epistaxis in summer was observed in one child each of sickle cell disease and sickle cell-β^{+}-thalassemia.

The distribution of huge splenomegaly can be graded into sickle cell carriers (1.6%) < sickle cell-β^{++}-thalassemia (7.1%) < sickle cell-β^{+}-thalassemia (10.0%) < sickle cell disease (10.3%) cases. Enlargement of liver (6.9%), aplastic crisis (6.9%), splenic sequestration (10.3%) and cardiac murmur (3.4%) were observed in sickle cell disease patients in India. Loss of weight was marked in both the sickle cell disease (17.2%) and sickle cell-β^{+}-thalassemia (10.0%) patients (Table 3). Retardation of growth and development was prominent in all patients. Menarche was delayed in both sickle cell disease (10.3%) and sickle cell-β^{+}-thalassemia (10.0%) patients.

History of hospitalization was recorded in sickle cell disease (10.3%), sickle cell-β^{+}-thalassemia (10.0%) and sickle cell-β^{++}-thalassemia (7.1%) cases (Table 3). Similarly, the history of repeated blood transfusion was prominently high in sickle cell disease (34.5%) and sickle cell-β^{+}-thalassemia (10.0%), in comparison to sickle cell-β^{++}–thalassemia (7.1%) patients.

Table 4 presents the summary sheet of haematological and clinical features of a sickle cell-Hb E-disease family in Orissa.

It is well established that the Indian population presents not only the socio-cultural and ethnic diversity but genetic heterogeneity also[7,12,14–16]. The history of India has witnessed admixture of so many foreign elements like Aryans and non-Aryans, Dravidians and Chinese, Scythians, Huns, Pathans and Moghuls that all are mixed, merged and lost in one body[12,14–17]. Therefore, an admixture of different thalassemia mutations is not at all surprising. Mild and severe forms of the hybrid disease occur whenever the sickle cell and β-thalassemia genes are found together in an individual in a particular locality, region or state of the country. The present study is from Orissa, where both the congenital sickle cell disease and β-thalassemia syndrome are highly prevalent[7,8,15,17] witnessing a hybrid scenario in central east coast of India.

The clinical course of sickle cell-β-thalassemia may range from one extreme, in which the condition is indistinguishable from severe sickle cell anaemia, to a disorder which is completely symptomless and, which may be found incidently during a family study or population

Table 4. Summary sheet of haematological and clinical features of sickle cell-Hb E disease in a Khandayat family from Khurda district in Orissa, India

	Parents		Offspring
Parameters of study	Father	Mother	Son
Age in years	27	23	1
Sex	Male	Female	Male
Haematological indices			
Hb (g/dl)	11.2	12.6	9.4
RBC ($\times 10^6$/µl)	4.5	3.4	3.6
HCT (%)	35.3	15.4	28.2
MCV (fl)	69.0	61.9	60.9
MCH (pg)	24.6	31.0	30.0
MCHC (g/dl)	27.5	19.3	19.3
RDW (%)	8.6	13.4	11.4
WBC ($\times 10^3$/µl)	6.3	12.9	4.7
Sickling test	–ve	+ve	–ve
Electrophoresis			
Major bands	AA_2	SE	AEF
Hb A_2 (%)	6.6	5.7	5.7
Hb F (%)	1.4	4.8	7.3
Hb E (%)	–	20.5	60.0
Hb S (%)	–	69.0	–
Hb A (%)	92.0	–	27.0
Clinical features			
Spleen (cm)	–	–	2
Liver (cm)	–	2	3
Symptoms	–	History of jaundice	Pallor, recurrent fever, transfusion dependent

screening, or presenting as a mild, refractory anaemia in pregnancy. The milder forms of disease, most common in African populations, have been well documented in Jamaica or elsewhere[3], whereas, the severe forms or many different β-thalassemia mutations (Mediterranean) involved, make it much more than an anecdotal account of the disease, witnessed by historical evidence and supported by the present study in India. The action of β-thalassemia mutation modifies the severity of the sickle cell-β-thalassemia genotype, compared with that of sickle cell anaemia, i.e. in case of β^+-thalassemia alleles, it allows the production of Hb A, while overall it reduces the absolute level of Hb S[3].

The level of Hb A in patients with sickle cell-β-thalassemia depends on the particular β-thalassemia mutation. The promoter mutations in Africa cause a mild reduction in β-chain production and are associated with average 20% level of Hb A resulting in a mild disease.[3] On the other hand, Mediterranean mutations have an average 10% level of Hb A resulting in severe disease. The findings of the present study are also consistent with severe sickle cell-β-thalassemia disease in India. At the other end of the spectrum are the patients inheriting β^0-thalassemia mutation with no Hb A but very similar to sickle cell anaemia[3].

The factors responsible for high level of Hb F in sickle cell-β-thalassemia are still not well understood. Raised level of Hb F condition provides selective survival of red cells and inhibiting effect on the intracellular sickling. The most striking evidence for the importance of high level of Hb F production in this disease is seen in the populations of Saudi Arabia, Iran and Orissa (India), where, despite the inheritance of severe β-thalassemia alleles, the disease is relatively mild[18,19]. The findings of the present study are consistent with this notion (Table 2). Contrary to Hb F linkage with the haplotype, family studies revealed that there is no linkage of sickle cell haplotype with the elevated level of Hb F, because carriers (single dose, Hb S) of sickle cell disease do not show any raised level of Hb F in sickle cell trait in the present study. When the Hb S appears in double dose (Hb SS) or in combination with β-thalassemia, only then the promoter region elevates the Hb F to compensate the reduction due to Hb S/β-thalassemia. Sickle cell trait subjects were found symptomless or showed mild clinical manifestations, if any, in the present study.

Clinically, more severely affected homozygous sickle cell disease individuals tend to experience sickle cell anaemia early in life. Joint pains accompanied by hand and foot syndrome in early infancy and childhood are the most common presenting symptoms of sickle cell anaemia, followed by osteomyelitis, splenic infarction, jaundice and pneumonia (Table 3). Painful crises of joint pains occur in rainy and cold weather in India. Stunted or

retarded growth, short stature and lighter body with bossing of the skull are frequently seen features. Huge solidified enlargement of spleen, requiring splenectomy and palpable liver are usual. As in sickle cell anaemia; there is usually a mild degree of cardiac enlargement with soft systolic murmurs due to chronic anaemia in sickle cell-β-thalassemia also. Leg ulceration and priapism are uncommon in Indian sickle cell disease and sickle cell-β-thalassemia patients (Table 3).

The Hb A level in three cases of sickle cell-β^+-thalassemia in the present study was observed to be 5.5%, 6.5% and 7% compensated by the inverse relationship with high level of foetal haemoglobin, i.e. 38.1%, 19.7% and 11.6% respectively, showing the variability of severity of clinical manifestations, haematological profile as well as genetic heterogeneity of the sickle cell-β-thalassemia in India[12,20].

The severity of sickle cell anaemia is variable ranging from life threatening complications and death in early childhood at one end of the spectrum to transient symptoms or a relatively benign clinical course at the other end[11,13]. Clinical examination of the homozygous sickle cell disease patients showed that splenomegaly was present (Table 3). The common clinical symptoms were moderate to severe anaemia, recurrent fever, intermittent mild to moderate jaundice, marked stunting growth, repeated vaso-occlusive crises, recurrent upper respiratory tract infection, severe pain in joints (knee, elbow, ankle), abdomen, chest which warranted hospitalization[13,21–23]. Thus, the findings of the present study suggest that the sickle cell patients sharing identical genotypes exhibited considerable heterogeneity in haematological features as well as in clinical symptomatology (Table 3). However, further molecular analysis of the sickle cell-β-thalassemia in the Indian populations is emphasized.

The community or population diversity of sickle cell haemoglobinopathy in India is most certainly caused by the admixture of genes between different intruders or invading groups in and around the Gulf region and India, Middle Eastern countries and Africa[6,13,14,22]. The gene flow and heterogeneity of β-thalassemia mutations represent complex anthropological influences from the East Mediterranean, Asia, sub-Sahara and east Africa corroborating that the community diversity of sickle cell and β-thalassemia mutations reflects historical events and gene migration in the region[12,14]. The β-thalassemia distribution, heterogeneity of mutations, homozygous births due to consanguinity, founder effect compounded with cultural traditions and beliefs are some of the important factors that determine the propensity of sickle cell phenotypes and β-thalassemia mutations in India.

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