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Prevalence of Alpha Thalassaemia in Sickle cell Disease of Jabalpur and its surrounding Districts of Madhya Pradesh

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ABSTRACT

A total of 254 samples of homozygous sickle cell anaemia (SCA) and 71 of sicke β-thalassaemia (SBT) from Jabalpur and its surrounding districts of Madhya Pradesh are screened for α^+-thalassemia mutations ($-\alpha^{3.7}$ & $-\alpha^{4.2}$ deletion). The overall prevalence of alpha thalassaemia (α-thalassaemia) is 39.4% in SCA and 40.8% in SBT while 39.7% in general. Heterozygous α-thalassaemia ($-\alpha/\alpha\alpha$) and $-\alpha^{3.7}$ deletions are predominated in both categories (SCA and SBT). The Scheduled tribe (ST) populations of SCA (60.0%) and SBT (100.0%) are found to have higher prevalence of α-thalassaemia followed by other backward class (OBC) group (54.2% in SCA & 44.1% in SBT). The heterozygous ($-\alpha/\alpha\alpha$) are higher in OBC (42.3%) of SCA and ST (57.2%) of SBT while homozygous ($-\alpha/-\alpha$) are higher in ST of both categories (22.9%in SCA and 42.9% in SBT). The $\alpha^{3.7}$ deletion is also higher in ST of both categories (48.6% in SCA and 85.8% in SBT). The $-\alpha^{4.2}$ deletion is 13.5% in OBC followed by ST (11.4%) among SCA while 18.7% in others followed by OBC (14.7%) among SBT. The significantly lower mean MCV, MCH and MCHC values and significantly higher total mean TRBC are observed in α-thalassaemia group of SCA. The homozygous α-thalassaemia ($-\alpha/-\alpha$) group of SCA have significantly lower mean MCV and MCH than heterozygous α-thalassaemia ($-\alpha/\alpha\alpha$). No any significant difference has been observed in mean haematological parameters between α-thalassaemia and without α-thalassaemia subjects of SBT.

Key word: *Alpha thalassaemia, Sickle cell anaemia, Sickle beta thalassaemia, Madhya Pradesh.*

INTRODUCTION

Sickle haemoglobin (HbS) is caused by a single nucleotide substitution (A → T) in the 6th codon of β-globin gene on chromosome 11 which results in replacement of Glutamic acid (GAG) by Valine (GTG) at the 6th position of the β-globin chain.[1,2] In deoxygenation conditions, this substitution causes HbS polymerization[3] and this mutation modify the stability of the haemoglobin and leads to the clinical disorder. The homozygous state of the sickle gene (β^S) results in sickle cell anemia (SCA) and is responsible for the most common severe variant of Sickle cell disease. Sickle cell disease (SCD) show a notable diversity in the severity of disease and Alpha thalassaemia is the one of the major modifiers in Sickle cell disease (SCD).[4] Alpha thalassaemia is classified into the **α^0-thalassaemia** in which synthesis of α-chains is absent and the **α^+-thalassaemia** in which synthesis of α-chains is reduced.[5] The α^+-thalassaemia is commonly caused by the deletion of one of the linked pairs of α-globin genes. The common deletions which cause α^+-thalassaemia are $-\alpha^{3.7}$ deletion (rightward deletion) and $-\alpha^{4.2}$ deletion (leftward deletion) which remove one or more of the duplicated structural α-genes.[6,7] The frequency of α^+-thalassaemia is higher (10–20%) in the parts of sub-Saharan Africa, more than 40% in Middle Eastern while it is up to 80% in north-east India and northern Papua New Guinea.[8] These frequencies show a strong correlation with malarial endemicity.[9] The present study is carried out to see the prevalence of α^+-thalassemia mutations ($-\alpha^{3.7}$&$-\alpha^{4.2}$) in the sickle cell disease subjects and its effect on red cell indices in Jabalpur and its surrounding districts of Madhya Pradesh.

METHODLOGY

A total of 325 samples were screened for the α-thalassemia deletions in sickle cell disease (SCD) individuals with their consent. *Homozygous Sickle cell anaemia* (254) and S*icke β-thalassaemia* (71) individuals have been included in this study. All these subjects are referred from various OPD's of NSCB Medical College, Jabalpur to Regional Medical Research Centre for tribals for the diagnosis of haemoglobinopathies. Diagnosis of these subjects is established by investigation of their Parents. If both parents are found to be heterozygous for sickle haemoglobin, their child is considered as homozygous sickle cell disease (*sickle cell anaemia-SCA*). If one parent is heterozygous for sickle haemoglobin and other parent has normal haemoglobin pattern with elevated HbA_2 levels, their child is considered as double heterozygous for sickle gene and thalassaemia gene (*sickle beta thalassaemia-SBT*). Hematological parameters (Hb%, MCV, MCH and MCHC) are measured on an automated blood cell counter (Cellenium 19). Sickle haemoglobin is identified by sickling test with 2% sodium metabisulphite and confirmed by electrophoresis which is done on cellulose acetate membrane with TEB buffer (Tris-EDTA-Borate) at pH 8.6(Fig.1).[10] HbA_2 is quantified by column chromatography to diagnose the β-thalassaemia trait.[11] DNA has been extracted by the Fermentas genomic DNA purification kit (K0512) and is screened for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions by

PCR(Fig.2 & Fig.3).[12] The statistical calculation used is Student's t-test with SPSS windows version 11.0.

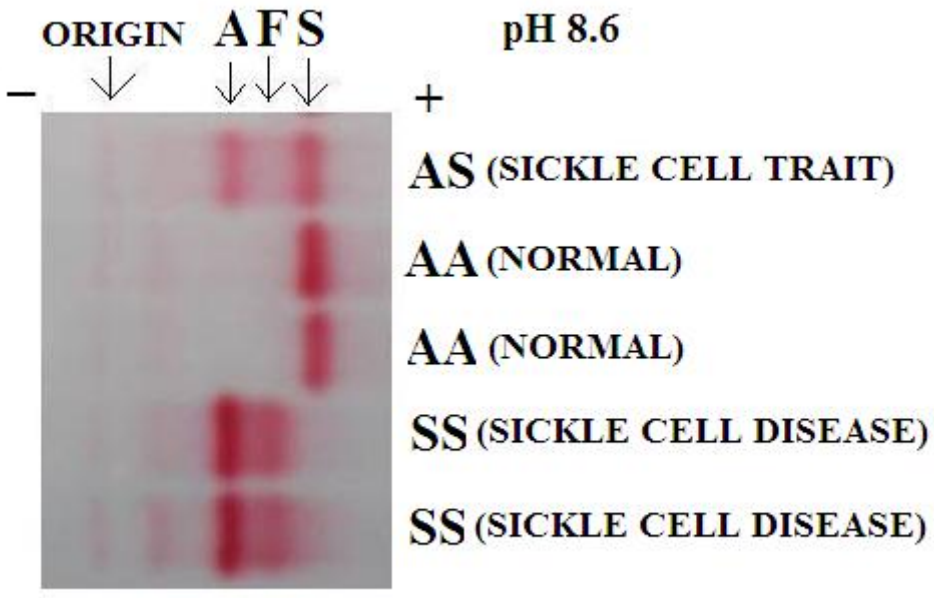


Fig.1- Haemoglobin electrophoresis on cellulose acetate medium

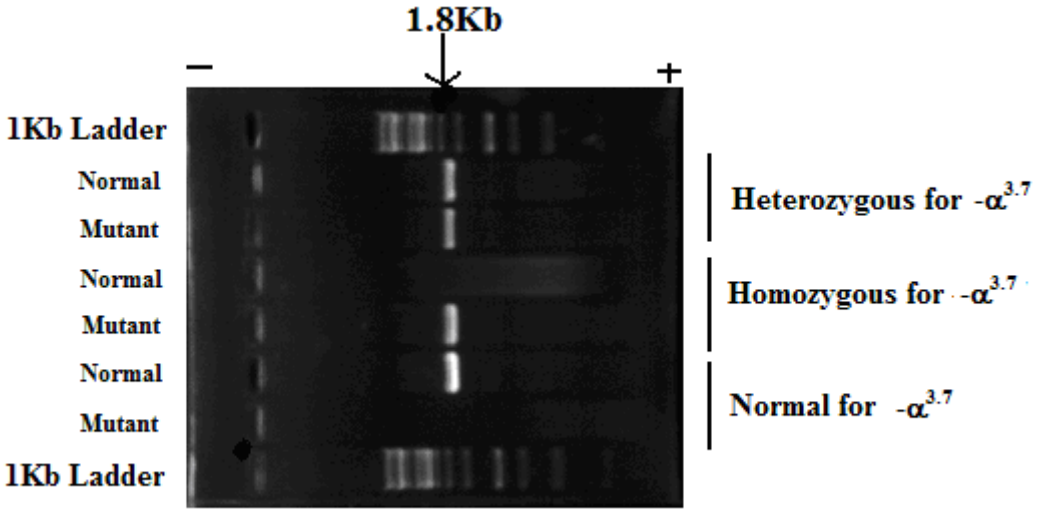


Fig. 2-Agarose gel electrophoresis of PCR product to detect -$\alpha^{3.7}$ deletion

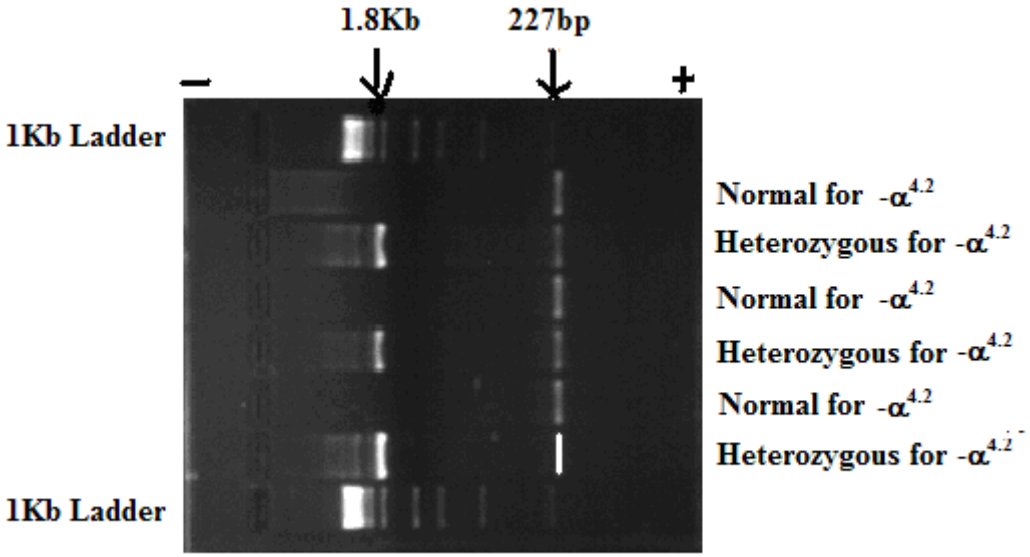


Fig. 3-Agarose gel electrophoresis of PCR product to detect -$\alpha^{4.2}$ deletion

RESULTS

Molecular analysis of two hundred fifty four *homozygous sickle cell anaemia* (SCA) and seventy one *sickle beta thalassaemia* (SBT) subjects is carried out for -$\alpha^{3.7}$ and -$\alpha^{4.2}$ deletions. Prevalence α-thalassaemia in SCA and SBT subjects of Jabalpur and surrounding districts are given in Table-1. About 49.5% subjects belong to Jabalpur district while 50.5% are from surrounding districts. The overall prevalence of α-thalassaemia is 39.7% (129/325) in general (SCA and SBT). The majority (30.8%) are found with heterozygous α-thalassaemia (-α/αα) and 7.7% have homozygous α-thalassaemia (-α/-α). All the homozygous α-thalassaemia subjects of our study are homozygous for -$\alpha^{3.7}$deletion. Homozygous for -$\alpha^{4.2}$deletion are not found. The prevalence of -$\alpha^{3.7}$ deletion is higher (29.5%) than -$\alpha^{4.2}$ deletion (11.4%). About 1.2% are found with double heterozygous for both deletions (-$\alpha^{3.7}$/-$\alpha^{4.2}$).

Homozygous sickle cell anaemia (SCA): About 48.4% (123/254) belonging to Jabalpur district and 51.6% (131/254) are from different surrounding districts. The prevalence of α-thalassaemia in SCA is 39.4% (100/254). The majority (29.5%) are with heterozygous α-thalassaemia (-α/αα) and 8.7% are with homozygous (-α/-α). The prevalence of -$\alpha^{3.7}$ deletion is higher (30.4%) than -$\alpha^{4.2}$ deletion (10.2%) while 1.2% are with double heterozygous for both deletions (-$\alpha^{3.7}$/-$\alpha^{4.2}$) in SCA.

The prevalence of α-thalassaemia in SCA is 43.1% (53/123) in Jabalpur district. The majority (32.6%) are with heterozygous α-thalassaemia (-α/αα) and 8.9% are homozygous (-α/-α). The -$\alpha^{3.7}$ deletion is foun to be higher (32.5%) than -$\alpha^{4.2}$

deletion (12.2%) while 1.6% are found with double heterozygous for both deletions (-$\alpha^{3.7}$ /-$\alpha^{4.2}$). About 35.9% (47/131) of surrounding districts were found with α-thalassaemia. The majority (26.7%) are found with heterozygous α-thalassaemia (-α/αα) and homozygous (-α/-α) is 8.4%. The prevalence of -$\alpha^{3.7}$ deleion is higher (28.3%) than -$\alpha^{4.2}$ deletion (8.4%) and 0.8% of individuals are found with double heterozygous for both deletions (-$\alpha^{3.7}$/-$\alpha^{4.2}$).

Sickle beta thalassaemia (SBT): About 53.5% (38/71) belong to Jabalpur district and 46.5% (33/71) are from surrounding districts. The prevalence of α-thalassaemia is 40.8% in SBT population. In SBT also, majority (35.2%) are with heterozygous α-thalassaemia (-α/αα) and 4.2% are homozygous (-α/-α). Here also, the prevalence of -$\alpha^{3.7}$ deletion (26.7%) is higher than -$\alpha^{4.2}$ deletion (15.5%) and 1.4% are found with double heterozygous for both deletions (-$\alpha^{3.7}$ /-$\alpha^{4.2}$).

About 34.2% (13/38) of SBT from Jabalpur district are found to have α-thalassaemia. The heterozygous α-thalassaemia (-α/αα) is higher (28.9%) than homozygous (-α/-α) i.e. 5.3%.The-$\alpha^{3.7}$deletion (23.7%) predominates over -$\alpha^{4.2}$deletion (10.5%) and subjects with double heterozygous for both deletions (-$\alpha^{3.7}$ /-$\alpha^{4.2}$) are not found. About 48.5% (16/33) of SBT from surrounding districts are found with α-thalassaemia. The majority (42.5%) are with heterozygous α-thalassaemia (-α/αα) and 3.0% are homozygous (-α/-α).The -$\alpha^{3.7}$ deletion is also higher (30.3%) than -$\alpha^{4.2}$ deletion (21.2%) and 3.0% are found with both deletions (-$\alpha^{3.7}$ /-$\alpha^{4.2}$).

The prevalence of α-thalassaemia are further analyzed according to Caste (Table-2).

Homozygous sickle cell anaemia (SCA): About 59.5% are belonged to Scheduled caste (mainly Jharia, Deharia, Mehra, Mahar, Katiya, Chadar etc.) followed by 23.2% of Other backward class (mainly Patel, Yadav and Barman etc.), 13.8% of Scheduled tribe (mainly Gond and Pradhan etc.) and 3.5% of others (Muslim and Rajpoot). The prevalence of α-thalassaemia in SCA is higher (60.0%) in Scheduled tribe (ST) followed by 54.2% in other backward class (OBC), 29.8% in Scheduled caste (SC) and others (22.2%). The heterozygous α-thalassaemia (-α/αα) is higher in OBC (42.3%) followed by ST (37.1%), SC (23.2%) and others (22.2%). The homozygous α-thalassaemia (-α/-α) is higher in ST (22.9%) followed by OBC (11.9%), SC (4.6%) and nil in others. The prevalence of -$\alpha^{3.7}$ deletion in SCA is 48.6% among ST followed by OBC (40.7%), SC (22.5%) and Others (22.2%). The prevalence of -$\alpha^{4.2}$ deletion is 13.5% among OBC followed by 11.4% in ST and 9.3% in SC. Subjects with double heterozygous for both deletions (-$\alpha^{3.7}$/-$\alpha^{4.2}$) are found only in SC (2.0%).The -$\alpha^{4.2}$ deletion is not found in studied others.

Sickle beta thalassaemia (SBT): About 47.9% are belonged to Other backward class (mainly Patel, Yadav and Barman etc.) followed by 22.5% of others (Muslim and Rajpoot), 19.7% of Scheduled caste (mainly Jharia, Dahia and Choudhary), and 9.9% of Scheduled tribe (Gond). All the SBT

subjects of ST category are found to have α-thalassaemia followed by OBC (44.1%), Others (25.0%) and SC (21.4%). The heterozygous α-thalassaemia (-α/αα) is higher in ST (57.2%) followed by OBC (41.2%), others (25.0%) and SC (21.4%). The homozygous α-thalassaemia (-α/-α) in SBT is found only in ST category (42.9%). The prevalence of $-\alpha^{3.7}$ deletion in SBT is also higher in ST (85.8%) followed by 32.3% in OBC, 7.1% in SC and 6.3% in Others. The prevalence of $-\alpha^{4.2}$ deletion in SBT is found to be 18.7% in Others followed by 14.7 in OBC and 14.3% among SC as well as ST. Subjects with double heterozygous for both deletions ($-\alpha^{3.7}/-\alpha^{4.2}$) are found only in OBC group (2.9%).

Mean haematological profile of sickle cell disease cases with or without α-thalassaemia are given in Table-3. In ***homozygous sickle cell anaemia (SCA),*** Mean haemoglobin levels of both groups are in the range of moderate anaemia and no any significant difference has been observed in Mean haemoglobin levels and PCV of SCA. The mean MCV (78.7 ± 10.6 fl), MCH (24.6 ± 4.0 pg) and MCHC (31.1 ± 2.3 g/dl) of alpha thalassaemia group are significantly lower than the mean MCV (87.1 ± 8.9 fl), MCH (27.9 ± 4.1 pg) and MCHC (31.8 ± 2.7 g/dl) of non-alpha thalassaemia group. Significantly increased mean total red cell count ($3.2 \pm 0.8 \times 10^6$) was observed in alpha thalassaemia when compared with non-thalassaemia group. No any significant difference was observed in the mean HbF levels of both groups. The mean MCV (75.1±8.4 fl), MCH (23.2±3.4 pg) of homozygous alpha thalassaemia (-α/-α) are significantly lower than the mean MCV (79.9±11.0 fl), MCH (25.0±4.1 pg) of heterozygous (-α/αα) alpha thalassaemia (Table-4). ***In sickle beta thalassaemia (SBT),*** statistically significant difference in mean haematological parameters between subjects with α-thalassaemia and without α-thalassaemia is not noticed.

Table 1- Percent prevalence of α-thalassaemia in sickle cell disease

	District	No. of Patients	$-\alpha^{3.7}/-\alpha^{3.7}$	$-\alpha^{3.7}/\alpha\alpha$	$\alpha\alpha/-\alpha^{4.2}$	$-\alpha^{3.7}/-\alpha^{4.2}$	Total α-thal.
Homozygous sickle cell disease (HbSS)	**Jabalpur**	123	8.9	22.0	10.6	1.6	43.1
	Surrounding districts (Anooppur, Balaghat, Chindwara, Damoh, Dindori, Hoshangabad, Katni, Mandla, Narsingpur,Satna, Sagar, Seoni, Singroli, Umaria)	131	8.4	19.1	7.6	0.8	35.9
	Total	**254**	**8.7**	**20.5**	**9.0**	**1.2**	39.4
Sickle β-thalassaemia	**Jabalpur**	38	5.3	18.4	10.5	0	34.2
	Surrounding districts (Damoh, Dindori, Hoshangabad, Katni, Mandla, Narsingpur,Panna, Sagar, Satna, Seoni, Singroli)	33	3.0	24.3	18.2	3.0	48.5
	Total	**71**	**4.2**	**21.1**	**14.1**	**1.4**	**40.8**

Table 2-Percent prevalence of α-thalassaemia in sickle cell disease among different caste populations

	Caste	No. of Patients	$-\alpha^{3.7}/-\alpha^{3.7}$	$-\alpha^{3.7}/\alpha\alpha$	$\alpha\alpha/-\alpha^{4.2}$	$-\alpha^{3.7}/-\alpha^{4.2}$	Total α-thal.
Homozygous sickle cell disease (HbSS)	**SC**	151	4.6	15.9	7.3	2.0	29.8
	ST	35	22.9	25.7	11.4	0	60.0
	OBC	59	11.9	28.8	13.5	0	54.2
	Others	9		22.2	0	0	22.2
	Total	**254**	**8.7**	**20.5**	**9.0**	**1.2**	**39.4**
Sickle β-thalassaemia	**SC**	14	0	7.1	14.3	0	21.4
	ST	7	42.9	42.9	14.3	0	100.0
	OBC	34	0	29.4	11.8	2.9	44.1
	Others	16	0	6.3	18.7	0	25.0
	Total	**71**	**4.2**	**21.1**	**14.1**	**1.4**	**40.8**

Table 3-Mean haematological profile of α-thalassaemia in homozygous sickle cell anaemia and sickle β-thalassaemia

	Genotype	N	Hb (g/dl) (Mean±SD)	PCV (%) (Mean±SD)	TRBC (X 10^6) (Mean±SD)	MCV (fl) (Mean±SD)	MCH (pg) (Mean±SD)	MCHC (g/dl) (Mean±SD)	HbF (%) (Mean±SD)
Homozygous sickle cell disease (HbSS)	αα/αα	150	7.7±1.7	24.2±5.2	2.8±0.7	87.1±8.9	27.9±4.1	31.8±2.7	15.2±6.3
	α–thal	100	7.8±1.7	25.0±5.0	3.2±0.8	78.7±10.6	24.6±4.0	31.1±2.3	14.3±5.8
					P=0.000 95% CI	P=0.000 95% CI	P=0.000 95% CI	P=0.023 95% CI	
Sickle β-thalassaemia	αα/αα	40	7.3±1.7	24.0±5.6	3.6±0.9	67.4±7.6	20.5±2.8	30.5±2.2	15.9±5.7
	α–thal	28	7.8±2.1	25.0±6.1	3.7±0.9	68.1±6.3	21.1±2.3	31.1±2.2	14.0±5.0

Table 4-Mean haematological profile of hetero and homozygous α-thalassaemia in sickle cell anaemia (homozygous)

Genotype	N	Hb (g/dl) (Mean±SD)	PCV (%) (Mean±SD)	TRBC (X 10^6) (Mean±SD)	MCV (fl) (Mean±SD)	MCH (pg) (Mean±SD)	MCHC (g/dl) (Mean±SD)	HbF (%) (Mean±SD)
– α/αα	75	7.8±1.7	24.8±5.0	3.2±0.8	79.9±11.0	25.0±4.1	31.1±2.5	14.7±6.1
– α/ – α	25	7.9±1.7	25.5±4.9	3.4±0.8	75.1±8.4	23.2±3.4	30.9±1.9	13.2±4.6
					P=0.027 95% CI	P=0.039 95% CI		

DISCUSSION & CONCLUSION

About one third of sickle cell anemia patients have coexisting alpha thalassemia which is a result of heterozygous or homozygous for the -$\alpha^{3.7}$ deletion.[13] About 17.5% of Brazil, 36% of Southern California and 44% of Guadeloupe sickle cell anaemia patients were found with α-thalassaemia.[14,15,16] In a study of 60 sickle cell anaemia patients in AIIMS, 18 (30.0%) patients were found with α-deletions.[17] In Western India, all the sickle homozygous tribals (Valsad) and 32.0% of sickle homozygous non-tribal individuals (Nagpur) were found with α-thalassaemia.[18] In our sickle cell subjects of Madhya Pradesh, 39.4% of sickle cell homozygous (SCA) individuals and 40.8% of sickle β-thalassaemia (SBT) subjects are found with α-deletions. Alpha thalassaemia is higher in scheduled tribes of both categories (SCA & SBT). About 60% of SCA and 100% of SBT individuals from scheduled tribe found with α-thalassaemia. Majority are heterozygous (-α/αα) for $\alpha^{3.7}$deletion and -$\alpha^{4.2}$deletion but homozygous for -$\alpha^{4.2}$deletion are not found. The -$\alpha^{3.7}$deletion is dominating over -$\alpha^{4.2}$ deletions. Sickle cell disease individuals with α^{+}-thalassaemia were associated with higher haemoglobin levels, lower mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and α-thalassaemia reduces the concentration of sickle haemoglobin (HbS) thereby polymerization of sickle haemoglobin which may reduce the degree of haemolytic anemia in these individuals so that α-thalassaemia may be an important genetic determinant of hematologic severity in sickle-cell disease because it reduces polymerization of sickle haemoglobin in homozygous sickle cell disease.[19-20] In India, co-inheritance of α-thalassaemia with sickle cell anaemia has been well studied in Orissa and western India. In Orissa, homozygous sickle cell (SS) disease patients with α-thalassaemia had higher levels of fetal haemoglobin, total haemoglobin, red cell counts and lower MCV, MCHC, reticulocyte counts when compared with Jamaican patients.[21] In a study of 42 SCD patients from Western India, homozygous sickle cell disease subjects with α^{+} thalassaemia showed significantly fewer complications and higher hemoglobin levels and red blood cell counts with lower MCV and MCH.[22] Western Indian sickle cell anaemia patients with associated α-thalassaemia in one study showed significantly higher haemoglobin (Hb), haematocrit (HCT), red blood cell counts (RBC) and HbA_2 levels but lower MCH, MCV and clinical presentation was also milder.[23] In our sickle cell homozygous subjects of Madhya Pradesh with α-thalassaemia also showing significantly higher mean red cell count (TRBC), lower MCV, MCH and MCHC but not showing any difference in mean haemoglobin levels. SCA subjects with homozygous -$\alpha^{3.7}$deletion (-α/-α) showed significantly lower MCV and MCH when compared with heterozygous (-α/αα) alpha thalassaemia. The subnormal MCV and MCH are indicative of red cell microcytosis and hypochromia respectively that is commonly observed in thalassaemias in which globin component formation of

haemoglobin is defective. So, concurrent α-thalassaemia with sickle cell disease may result in quantitative effect on the intracellular concentration of HbS and reduces the concentration of HbS thereby polymerization of sickle haemoglobin.

In β-thalassaemia, the reduced/absence of β-globin chains result in a relatively freely unbound α-globin chains which precipitate in erythroid precursors in the bone marrow that results in their premature death in turn ineffective erythropoiesis.[24] Concurrent alpha thalassaemia with beta thalassaemia may reduce the quantity of unbound α-globin chains there by reduction in the precipitation in the bone marrow. In one study on the effects of alpha thalassaemia on sickle-β^0thalassaemia in West Indies *sickle beta thalassaemia* patients, α-thalassaemia was shown to be associated with significantly higher haemoglobin levels and lower reticulocyte count. Alpha thalassaemia was associated with significantly higher MCV and MCHC and the effect of alpha thalassaemia on haematological and clinical parameters was less in sickle-β^0 thalassaemia than in hoimozygous sickle cell disease.[25] In other study, no differences were noticeable between SBT with α-thalassaemia and without α-thalassaemia in the haemoglobin levels, HbA_2, MCV and clinical features but fetal haemoglobin (HbF) levels were significantly higher in patients those without α-thalassaemia.[26] In our study, mean total haemoglobin levels, PCV, TRBC, MCV, MCH and MCHC of alpha thalassaemia group are seems to be slightly higher but statistically not significant. Further analysis of haematological data according to mutation responsible for the beta thalassaemia which is not done in this study may be helpful for the conclusion. Comparison of the clinical parameters of sickle cell disease with alpha deletions may give an idea on the effect of alpha thalassaemia on the severity of the disease in Madhya Pradesh and study may be initiated in that way further. It is concluded that more than one third of sickle cell disease individuals of Madhya Pradesh are carry the α-deletions which may possibly be an important genetic factor that may modulate the haematological severity as well as clinical expression and can be used as the predictor of disease severity in this area.

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REFERENCES

1. Pauling, L., Itano, H., Singer, S.J., and Wells, I.C. Sickle cell anemia: a molecular disease: Science 110: 543–548. 1949.
2. Ingram, V.M. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin**:** Nature 178: 792–794. 1956.

3. Bunn HF. Pathogenesis and treatment of sickle cell disease: N Engl J Med Sep 11;337(11):762–769. 1997.
4. Thein SL. Genetic modifiers of the b-haemoglobinopathies-review: British Journal of Haematology, 141, 357–366. 2008
5. Weatherall, D.J. & Clegg, J.B. The Thalassaemia Syndromes: Blackwell Science, Oxford. 2001.
6. Embury SH, Miller JA, Dozy AM, KanYW, Chan V, Todd D.1980. Two different molecular organizations account for the single α-globin gene of the α-thalassemia-2 genotype.J Clin Invest 66: 1319–1325. 1980.
7. Higgs DR. The molecular basis of α-thalassemia: Cold Spring Harb Perspect Med. 2013 Jan 1;3(1):a011718.2013.
8. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem: Bulletin of the World Health Organisation, 79: 704–712. 2001.
9. Flint J, Hill AVS, Bowden DK, Oppenheimer SJ, Sill PR, Serjeantson SW, Bana-Koiri J, Bhatia K, Alpers MP, Boyce AJ, Weatherall DJ and Clegg JB. High frequencies of α thalassaemia are the result of natural selection by malaria: Nature 321: 744–749. 1986.
10. Chanarin I. Laboratory Haematology: An account of Laboratory Techniques.1st edition Pub. by Churchill Livingstone, London. 1989.
11. Dacie JV and Lewis SM. Practical Haematology: Seventh Edition. Churchill Livingstone, London. 1981
12. Baysal E, Huisman THJ. Detection of common deletional α-thalassaemia-2 determinants by PCR: Am J Haematol 46: 208-213. 1994.
13. Steinberg MH. Genetic Etiologies for Phenotypic Diversity in Sickle Cell Anemia. Special Issue: Hemoglobinopathies. TheScientificWorld Journal 9: 46–67. 2009.
14. Figueiredo MS, Kerbauy J, Gonçalves MS et al. Effect of α-thalassemia and β-globin gene cluster haplotypes on the hematological and clinical features of sickle-cell anemia in Brazil: Am. J. Hematol. 53, 72–76. 1996.
15. Kéclard L, Ollendorf V, Berchel C et al. β^S haplotypes, α-globin gene status and hematological data of sickle cell disease patients in Guadeloupe: Hemoglobin 20: 63–74. 1996.
16. Schroeder WA, Powars DR, Kay LM et al. β-cluster haplotypes, α-gene status and hematological data from SS, SC, and S-β-thalassemia patients in southern California: Hemoglobin 13(4): 325–353. 1989.
17. Sanjay P, Sweta P, Rahasya MM, Monica S, Renu S. Genotypic influence of α-deletions on the phenotype of Indian sickle cell anemia patients: Korean J Hematol 46:192-195. 2011.

18. Mukherjee MB, Lu CY, Ducrocq R, Raman R. Gangakhedkar, Colah RB, Kadam MD, Mohanty D, Nagel RL and Krishnamoorthy R. Effect of α-Thalassemia on Sickle-Cell Anemia Linked to the Arab-Indian Haplotype in India: American Journal of Hematology 55:104–109. 1997.
19. Higgs DR, Aldridge BE, Lamb J et al. The interaction of α-thalassemia and homozygous sickle cell disease. N. Engl. Med. 306: 1441. 1982.
20. Embury SH, Dozy AM, Miller J et al. Concurrent sickle-cell anemia and α-thalassemia. Effect on severity of anemia: N. Engl. J. Med. 306:270. 1982.
21. Kar BC, Satapathy RK, Kulozik AE, Kulozik M, Sirr S, Serjeant BE, Serjeant GR. Sickle cell disease in Orissa State, India**:** Lancet. 2:1198-201. 1986.
22. Mukherjee MB, Colah RB, Ghosh K, Mohanty D and Krishnamoorthy R. Milder Clinical Course of Sickle Cell Disease in Patients With alpha Thalassemia in the Indian Subcontinent: Blood 89: 732. 1997.
23. Mukherjee MB, Surve R, Tamankar A, Gangakhedkar RR, Ghosh K, Lu CY, Krishnamoorthy R, Colah R, Mohanty D. The influence of alpha thalassaemia on the haematological and clinical expression of sickle cell disease in western India: Indian J Med Res. 107:178-181. 1998.
24. Galanello R, Origa R. Beta-thalassemia-Review**.** Orphanet Journal of Rare Diseases 5:11**.** 2010.
25. Vyas P, Higgs DR, Weatheral DJ, Dunn D, Serjeanat BE and Serjeant GR. The interaction of alpha thalassaemia and sickle cell-beta^0 thalassaemia: British Iournal of Haernatology. 70: 449-454. 1988.
26. Steinberg MH, Coleman MB. Adams JG and Rosenstock W. Interaction between HbS-beta^0-thalassemia and alpha thalassemia: American Journal of Medical Sciences, 288: 195-199. 1984.