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Microcytic red cell morphology in Dhodia Patel Community: Is there α-thalassemia?

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Introduction

Microcytic red cell indices are commonly associated with iron deficiency anemia (IDA) and β-thalassemia. If these causes are ruled out then there is a possibility of α-thalassemia [1].

α–thalassemia is caused by impaired production of α globin chains. The commonest α gene defect in the Indian population is the deletion of one or two genes. The severe and fatal form of α thalassemia, hemoglobin (Hb) Bart's hydrops fetalis is not reported in India. Few cases of Hb H disease have been reported [2]- [5]. α–thalassemia has a widespread distribution worldwide. The literature reveals that it is mainly limited to certain tribal groups with a prevalence of 8 to 81 % [6]- [7].

Dhodia Patel is the third largest tribal community in Gujarat. Majority of them are settled in the southern part particularly in Surat and Valsad districts. Our preliminary investigation on this community suggested that healthy subjects have low values of red cell indices in absence of β-thalassemia trait. Hence it was decided to rule out IDA and α-thalassemia in this community.

1.Methods

This study was approved by the institutional ethics committee. The blood samples of Dhodia Patel and controls (non-tribal) were collected from school and college students after taking their informed consent in Gujarati or Hindi language. Total 1155 Dhodia Patel and 4780 control subjects were investigated for hemoglobinopathies. Within 24 hours of collection the samples were run on automatic hematology analyzer. Samples showing mean corpuscular volume (MCV) ≤ 76 fL, mean corpuscular hemoglobin (MCH) ≤ 26 pg and Hb A_2 ≥ 3.5 % by cellulose acetate Hb electrophoresis [8] at pH 8.6 were diagnosed as β-thalassemia trait [9]. The samples showing solubility test positive results were further confirmed as sickle cell trait by Hb electrophoresis and Hb S level was estimated on spectrophotometer [10]. Serum ferritin concentration was measured by ELISA (FERRITINA IEMA WELL- RADIM) in 114 Dhodia Patel and 120 control (non-

tribal) samples negative for hemoglobinopathies but having MCV ≤ 76 fL, MCH ≤ 26 pg and Hb level > 10 g/dl. Value ≤15 ng/ml was considered as IDA [10]. Cellulose acetate Hb electrophoresis at pH 8.6 was carried out to detect Hb Bart's band in cord blood. Presence of Hb Bart's was confirmed on HPLC [11].

1.Statistics

Mean, standard deviation (SD), t-test and χ^2 were calculated using microsoft excel functions. α-gene frequency was calculated by Hardy-Weinberg equation:

$$\left(p^2\right)+\left(2pq\right)+\left(q^2\right)\right)=1$$

Where, P^2 = frequency of homozygous (- α/- α)

2pq = frequency of heterozygous (- α/α α)

q^2 = frequency of homozygous (α α/α α)

$$P=\frac{2\times obs\left(-\alpha\ /\ -\alpha\right)+obs\left(-\alpha\ /\ \alpha\alpha\right)}{2\times\left(obs\left(-\alpha\ /\ -\alpha\right)+obs\left(-\alpha\ /\ \alpha\alpha\right)\ +obs(\alpha\alpha/\alpha\alpha)\right)}$$

q = 1- p

1. **Results**

Table 1 shows the prevalence of β-thalassemia trait, sickle cell trait, sickle cell disease and other Hb variants detected in this study.

Table 1: Prevalence of hemoglobinopathies in Dhodia Patel and control subjects

Hemoglobinopathies	Dhodia Patel		Control	
	n	%	n	%
β-thalassemia trait	25	2.1	139	2.85
Sickle cell trait	162	14.0	59	1.21
Sickle cell disease	8	0.69	2	0.04
Other Hb variants	-	-	20	0.41
Total screened	1155		4780	

The hematological data of normal and sickle cell trait subjects in Dhodia Patel and control group are given in Table 2. This table shows that the normal subjects of this community have significantly reduced MCV and MCH and increased RBC count ($p<0.0001$). Similarly sickle cell trait subjects also have significantly

reduced MCV, MCH and Hb S % and increased Hb A_2 % compared to control group ($p<0.0001$). Further analysis suggested that 78.54% normal Dhodia Patel subjects had MCV values below 76 fL and 79.16% had MCH below 26 pg. In Dhodia Patels having sickle cell trait, MCV ≤ 76 Fl and MCH ≤ 26 pg was observed in 85.8% cases. In the control series MCV ≤ 76 FL and MCH ≤ 26 page was observed in 30.78% and 37.12% subjects respectively. Thus compared to Dhodia Patels control series showed significantly lower incidence of reduced red cell indices (χ^2=759 for MCV and 549 for MCH, $p<0.0001$).

Samples negative for hemoglobinopathies with MCV < 76 fl, MCH < 26 pg and Hb level > 10 g/dl were investigated for serum ferritin. Table 3 shows that 28.07 % Dhodia Patel and 60 % controls were having IDA. The incidence of IDA in control series having low red cell indices in absence of any hemoglobinopathies was significantly higher than that in Dhodia Patel community (χ^2= 24.1, $p<0.0001$).

Figure 1 shows the tri-modal distribution of Hb S, having peaks at 28, 31 and 33 in 162 sickle cell heterozygotes. On the basis of this graph, the rough assumption was made that the group having Hb S < 28 % is homozygous for α^+-thalassemia, with Hb S 28 to 33 % heterozygous and the group having Hb S > 33 % has normal α genotype. On this basis, the α-Gene frequency was calculated using Hardy-Weinberg equation. Table 4 shows high α-gene frequency of 0.73 in Dhodia Patels as compared to low (0.22) gene frequency in controls.

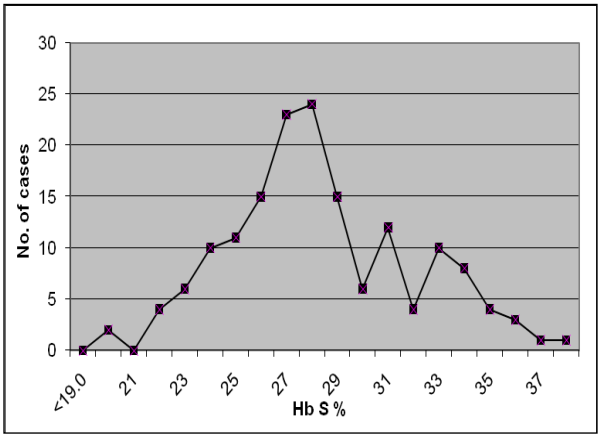


Fig 1: Distribution of Hb S levels in 162 Dhodia Patel sickle cell trait subjects

Table 2: Hematological parameters in Dhodia Patel and Control

Parameters	Normal		Sickle cell trait	
	Dhodia Patel (n=960)	**Control (n=4560)**	**Dhodia Patel (n=162)**	**Control (n=59)**
Hb (g/dL)	12.1 ± 1.73	13.1 ± 1.8	12.19 ± 1.49	12.4 ± 1.9
RBC(x 10^6/L)	**5.24 ± 0.73***	4.63 ± 0.63	5.34 ± 0.70	5.00 ± 0.8
HCT (%)	36.4 ± 5.2	39.3 ± 5.5	36.44 ± 4.54	37.6 ± 5.9
MCV (fL)	**69.8 ± 8.32***	85.5 ± 9.5	**68.68 ± 7.87***	75.1 ± 11.4
MCH (pg)	**23.6 ± 3.49***	29.2 ± 3.6	**23.07 ± 2.77***	25.1 ± 4.6
MCHC (g/dl)	33.7 ± 2.89	33.6 ± 2.9	33.56 ± 2.81	33.0 ± 3.07
RDW (%)	15.6 ± 2.03	14.7 ± 1.8	15.54 ± 2.28	15.8 ± 2.3
Hb A_2 (%)	2.67 ± 0.47	2.58 ± 0.5	**3.51 ± 0.69***	2.9 ± 0.8
Hb S (%)	-	-	**27.9 ± 3.4***	31.2 ± 5.8

***Statistically significant increase or decrease in mean values by "t" test (p <0.0001)**

Table 3: Ferritin levels in Dhodia Patels with microcytic morphology

Ferritin level	Tribal (Dhodia Patel)		Control	
	n	**%**	**n**	**%**
≤ 15 ng/ ml (IDA)	32	28.07	72*	60
> 15 ng/ml	82	71.9	48	40

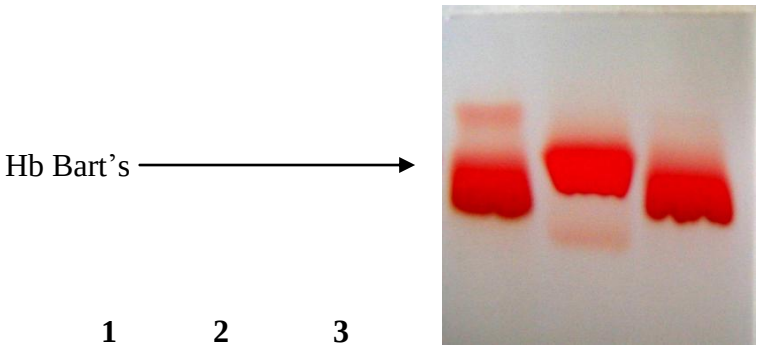
***Significantly higher incidence of IDA in control by $\chi 2$ test ($\chi 2$= 24.1, p<0.0001)**

Table 4: α-gene frequency in Dhodia Patel and Control

Genotype	Hb S %	Dhodia Patel n= 162	Control n=54
- α/- α - α/α α α α/α α	< 28 28-33 > 33	88 (54.32) 61 (37.65) 13 (8.03)	6 (11.11) 12 (22.22) 36 (66.67)
- α frequency		0.73	0.22
Projected α-thalassemia %		91.25	39.12

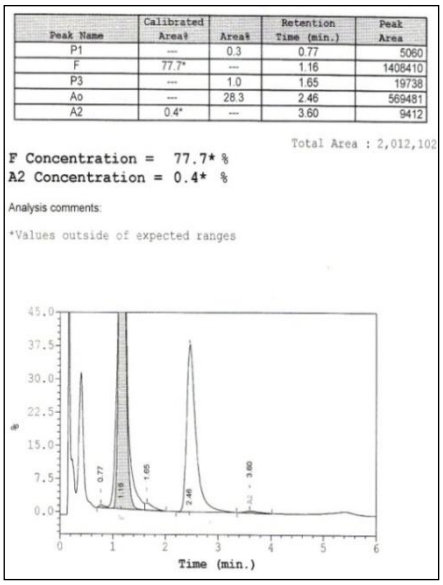
***Figures in parenthesis indicate % values**

Total 18 cord blood samples of Dhodia Patel and 20 non-tribal controls were screened for Hb Bart's. The cord blood having Hb Bart's shows fast moving band on cellulose acetate electrophoresis (lane 1, Fig 2) and sharp peak at the start of chromatogram on HPLC (Fig 3). The assumption was made that the samples having Hb Bart's < 2 % are normal, between 2-5 % are heterozygous for α-thalassemia and 5-8 % are homozygous for α-thalassemia. Table 5 shows that more than 75 % Dhodia Patel neonates had >2 % Hb Bart's level suggesting α-thalassemia in them. But the sample size was too small for statistical analysis.



Lane 1: Cord blood with Hb Barts
Lane 2: Normal adult sample
Lane 3: Cord Blood without Hb Barts

Fig 2 Cellulose acetate Electrophoresis (pH 8.6) showing fast moving band (Hb Bart's)



Peak Name	Calibrated Area‡	Area‡	Retention Time (min.)	Peak Area
P1	---	0.3	0.77	5060
F	77.7*	---	1.16	1408410
P3	---	1.0	1.65	19738
Ao	---	28.3	2.46	569481
A2	0.4*	---	3.60	9412

Total Area : 2,012,102

F Concentration = 77.7* %
A2 Concentration = 0.4* %

Analysis comments:

*Values outside of expected ranges

Fig 3: Chromatogram of cord blood sample with Hb Bart

Table 5: Distribution of Hb Bart's level in cord blood

Genotype	Hb Bart's (%)	Dhodia Patel n= 18	Control n=20

α α/α α	< 2	4	18
- α/α α	2-5	6	2
- α/- α	> 5	8	-

Discussion

The most common causes of microcytic red cell indices in Indian population are IDA and thalassemia. By proper measurement of red cell indices on electronic counter, suspected thalassemia carrier subjects can be identified using MCV and MCH values of ≤76 fL and ≤26 pg respectively. The Hb A_2 level >3.5 % confirms β-thalassemia trait [9]. Incidence of β-thalassemia trait and sickle cell trait in Dhodia Patels in our study is 2.1 % and 14.0 % respectively. We observed reduced MCV and MCH in majority (78.54% and 79.16% respectively) of normal subjects of this community which was significantly higher compared to control series. Even the sickle cell trait cases of this community had significantly lower values of MCV, MCH and Hb S %. Hence we suspected α-thalassemia in Dhodia Patel community.

Iron deficiency is diagnosed by low serum iron, low serum ferritin values, an elevated serum transferrin and a high total iron binding capacity (TIBC). To rule out IDA in Dhodia Patel subjects having microcytosis, we measured serum ferritin levels. Majority of them showed normal serum ferritin levels, while etiology of microcytic red cell indices in control series was IDA. Serum ferritin can be elevated due to chronic inflammation hence it may not be reliable parameter of iron status particularly if within normal limits [12]. Serum ferritin test is meaningful if levels are abnormally low.

α-thalassemia may also have reduced red cell indices therefore it is necessary to rule it out in our population, particularly in tribal communities. Studies on α-thalassemia in Gujarat tribes are available in the literature [13]- [16] but a systematic study is not available in Dhodia Patel community.

The heterogeneous distribution of Hb S levels has been explained by a genetic model based on the number of α –gene loci which can modify the net synthesis of Hb S [17], leading to a decreased in Hb S synthesis due to the lesser affinity of β^S chains for α –chain [18]. As reported earlier [19], [20], our study also found significantly lower Hb S % in Dhodia Patel (27.9 ±3.4) compared to control (34.02 ±4.2). On the basis of Hb S tri-modal graph, assumptive α-gene frequency and prevalence were calculated by Hardy Weinberg equation. The α-gene frequency was 0.73 and prevalence 91.25 % in Dhodia Patel which was high compared to controls.

The simple approach to screen α-thalassemia in mass population is cord blood screening for Hb Bart's, which can be identified by level of fast moving Hb Bart's electrophoresis band and a sharp peak at the start of the chromatogram on HPLC (11) [11]. This study found 14 Hb Barts cases among 18 cord bloods of Dhodia Patel. In controls only two samples were having Hb Barts. Though our sample size was small because of the difficulties faced in collecting cord blood at the time of delivery of women of Dhodia Patel community, results clearly support the observation of high prevalence of α-thalassemia in Dhodia Patel. However molecular study is required for confirmation.

Majority of Dhodia Patel normal and sickle cell trait subjects have microcytic red cell indices. Serum ferritin estimation to diagnose IDA in them shows that majority of them do not suffer from IDA. The cord blood screening for Hb Barts shows the evidence of α-thalassemia in this community but confirmation by molecular study is essential.

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