International Journal on Biological Sciences 7 (1) : 20-27, January-June 2016
ISSN : 0976-4518

Research Paper

Studies on The Prevalance of Hemoglobinopathies and Thalassemia Among Microcytic Hypochromic Anemia Cases in Metropolitan City of Chennai, Tamilnadu, India

BHAVANI, D. AND S. RAJAN

PG and Research Department of Zoology, Pachaiyappa's College, Chennai, Tamil Nadu

Received: 08 February 2016; **Revised:** 22 February 2016 **Accepted:** 28 March 2016

ABSTRACT

Microcytic hypochromic anemia is an accurate marker for the diagnosis of various hemoglobinopathies and thalassemia. It is proposed to study the prevalence of hemoglobinopathies and thalassemia among microcytic hypochromic anemia (MCHC) cases in the population of Chennai. A total of 996 blood samples were collected during the period of April 2014 to May 2015 which was referred for complete blood count by the physician. These samples were used for assessing various hemoglobinopathies and thalassemia for the present investigation using Bio rad D10^{TM} system-High Performance Liquid Chromatography (HPLC). In this present study, among 996 cases, 675 females and 321 males were noted. Out of 996 cases, 274 cases displayed abnormal hemoglobin fractions on HPLC. Among 274 cases, 221 cases were beta thalassemia trait, 3 beta thalassemia intermedia, 6 beta thalassemia major, 4 sickle cell trait, 1 case of HbS /beta thalassemia, 7 & 13 cases of HbE homozygous and heterozygous, 2 cases of HbE / beta thalassemia, 3 cases of HbD, 1 case of HbH, 3 cases of Delta thalassemia, 9 cases of borderline HbA2 and 1 case of Hb Lepore. 722 cases with normal and decreased HbA2 were considered for alpha thalassemia/Anemia. Generally microcytosis and hypochromia are often interpretated as indicators for iron deficiency. In the light of present investigation it is inferred that high prevalence of hemoglobinopathies and thalassemia cases were found with hypochromic microcytic anemia in Chennai.

Key words : Hemoglobinopathies, Hypochromic Microcytic anemia, Mean Red Cell Volume, Mean Red Cell Hemoglobin, Thalassemia.

INTRODUCTION

Hemoglobinopathies and thalassemia are inherited disorders causing severe microcytic hypochromic anemia (Weatherall and Clegg, 1981). Thalassemia and some of the Hemoglobin (Hb) Variants are inherited recessive, whereas very rarely Beta thalassemia is inherited as autosomal Dominant (Trent, 2006). Atleast 7.0 % of the world population is a carrier of hemoglobin disorder. 300,000- 500,000 children are born with clinically significant hemoglobin disorders annually. 50,000-100,000 children with thalassaemia major die each year in low and middle income countries (WHO-TIF, 2008). About 1.1% of couples worldwide are at risk for having children with hemoglobin disorder and 2.7 per 1000 conceptions are affected (Modell and Darliason, 2008 and Philip *et al.*, 2013). The carrier frequency of hemoglobinopathy varies between 3 and 17 % in different population in India (Balgir, 2000). In India beta thalassemia ranges from 1% to 17% with average of 3.2 % in general population (Kumar *et.al.*, 2015). The cumulative gene frequency for abnormal hemoglobin like sickle cell, hemoglobin D and E have been found to be 5.35% in India (Balgir, 2000). In India prevalence of alpha thalassemia varies from one geographical area to another: 42% to 71% in Orissa, 11% in Andhra Pradesh, 95 % in West Central Gujarat and 85.7% in Nilgiris of South India (Balgir, 2000) suggesting that the condition is almost genetically

Corresponding author: drsrajan@gmail.com

fixed in India (Lobie *et al.*, 1989). Hemoglobinopathies and Thalassemia are non-communicable genetic disorders in India causing moderate to severe hemolytic anemia which leads to several deaths in India (Balgir, 2005; Nadeem *et al.*, 2012; Pant *et al.*, 2014). In India, many researchers attempted for screening hemoglobinopathies and thalassemia among microcytic hypochromic anemia cases (Philip, *et. al.*, 2013). Hence it has been proposed to study the prevalence of hemoglobinopathies and thalassemia among microcytic hypochromic anemia cases in Metropolitan Chennai, TamilNadu.

Genetic background

Hemoglobin comprises four globin chains: two alpha chains and two beta chains (α 2 β2). Fetal hemoglobin (HbF) has two alpha and two gamma chains (α2 γ2), Adult hemoglobin (HbA) has two alpha and beta chains (α2 β2) and HbA2 has two alpha chains and two delta chains (α2 δ2). Expression of alpha globin and beta globin chains is regulated by cluster of genes on chromosomes 16 and 11 (Higgs, 2013). The globin moiety of Hemoglobin molecules is made of 7 different polypeptide chains and they are designated by the Greek letters alpha (α), beta(β), gamma (γ),delta (δ), episilon (ε) and zeta (ζ). The alpha and zeta chains contain 141 amino acid, whereas beta, gamma, delta and epsilon chains contain 146 amino acid. Epsilon, zeta and alpha are synthesized in early embryonic life. Alpha and gamma are synthesized in fetal life and alpha and delta in post natal life (Balgir, 2000).

In the earliest embryonic (fetal) life, zeta and epsilon (ζ 2 ε 2) chains combine to form HbGower 1, alpha and epsilon chains (α2 ε2) combine to form Hb Gower II and zeta and gamma chains form Hb Portland (ζ2 γ2). By the end of the first trimester zeta replaced by alpha chains and epsilon is replaced by gamma chains. In the late fetal life, HbF (α2 γ2) forms about 80 – 90% of total hemoglobin at birth, whereas in the post natal life only traces are present up to 6 – 12 months and fetal Hb is replaced by human Adult Hemoglobin HbA (α2 β2) comprising about 97% and HbA2 (α2 δ2) constitutes about 1.5 – 3.5% of total adult Hb (Balgir.2000).

Due to spontaneous mutation like chromosomal rearrangement, telomere truncations, homologous and illegitimate recombination, gene conversion, copy number variation, nucleotide polymorphism, changes in tandem repeats, abnormal methylation, the involvement of antisense RNA (Higgs, 2013) and segmental duplications and deletions (Lupski. *et al.*, 2005) are known to cause globin gene disorders which leads to hemoglobin gene variants which further get classified into two broad groups: Structural variants that change aminoacid sequence and produce an abnormal hemoglobin (qualitative) like Hb D, E, H, J , K, L, M, Q, S, Lepore, Norfolk, Koya Dora, Chandigarh and hereditary persistence of fetal hemoglobin (HPFH) and thalassemia -lower or abolish globin chain production (quantitative) - α thalassemia, β thalassemia. The most common such variants are alpha plus thalassaemia (Balgir, 2000).

MATERIALS AND METHODS

Blood samples were collected from 996 individuals who were prescribed for Complete Blood Count in Hitech Diagnostic Centre, Chennai and various centres of Chennai (mixed population) during the period of April 2014 to May 2015.

The complete blood count was performed using Sysmex XN 3000 Analyzer (Sysmex Corporation Kobe, Japan). Samples with MCV ≤ 80fl and MCH ≤ 27 pg were taken and further quantified for HbA2 using BioRad D10™ HPLC hemoglobin Testing System (D10 HbA2/F/A1c Dual program), that utilized the principle of high performance liquid chromatography (HPLC) a sensitive and precise method for quantification of HbA2, HbF and other abnormal hemoglobin fraction which includes Hb A, F, S, C, E/A2, D-Punjab, G- Philadelphia, D Arab. Such variant hemoglobin can be distinguished from each other based on the retention time (British Journal of Hematology, 1998 and Kumar *et al.*, 2015). Red cell morphology was analyzed using Peripheral smear. HbH Inclusions body test was done for the suspected cases. Consents were obtained from the patient including the history of blood transfusion and ethnicity.

Microcytosis and hypochromia results from deficient hemoglobin synthesis in erythroid cells, causing a reduction in both Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) of red blood cells (Weatherall and Clegg. 1981; Borges *et al.*, 2001 and Bain, 2004) The conventional diagnosis for thalassemia carriers is usually based on measurement of the low Mean Red Cell Volume (MCV) and Mean Red Cell Hemoglobin (MCH) (Kiss

et al., 2001; Trent, 2006 and Philip *et al.*, 2013). Beta thalassemia carriers and other hemoglobinopathies variants have been confirmed by automated HPLC which is a highly reliable technique with good resolution and it provides accurate quantification of hemoglobin fractions (Fucharoen and Winichagoon, 2011; Urrechaga, 2011 and Philip *et al.*, 2013). Prior approval is taken from institutional human ethical committee for this study (HDC/IHEC/03).

RESULTS AND DISCUSSION

In this study, a total number of 996 cases of microcytic hypochromic anemia were screened for beta thalassemia and other hemoglobinopathies and the results are presented in Table-1. Out of total 996 cases, 321 males and 675 females with age groups between 3 months to 85 years were observed. Among 675 females, about 389 cases were prenatal cases included in this study. Further, out of total 996 number of cases, 722 cases with normal and possible alpha thalassemia cases were observed. Further, about 30 cases of < 1 year, 139 cases of age < 12 years (children), 72 cases of Adolescent age (12-19 years), 489 cases of Adult age (20-40 years), 219 cases of Post Adult age (41-60 years) and 47 cases of Old age (> 60 years) were found. The distribution of normal chromatogram and chromatogram with hemoglobin variant among different age groups in the present investigation are presented in Table 2.

Table 1: Gender Distribution, Hb Fractions on HPLC and RBC indices among MCHC Anemia cases. n=996

Presumptive HPLC Diagnosis	Male	Female	No. of cases in %	HbF	HbA0	HbA2	RBC	Hb	HCT	MCV	MCH	MCHC	RDW-CV	Ab-normal variant
				Mean±SD	%±SD	%±SD	%±SD	%±SD	%±SD	%±SD	%±SD	%±SD	%±SD	
Suspected Alpha Thal/ Anemia	213	509	722 (72.49%)	0.92± 0.56	84.37± 3.55	2.09± 0.40	4.58± 1.72	8.58± 2.21	30.02± 6.16	66.40± 6.16	18.96± 3.64	28.26± 2.92	19.69± 3.86	
B Thal	83	138	221 (22.19%)	1.37±1.27	81.5 ±2.62	5.33±0.78	5.26±0.91	10.21±1.74	33.63±5.65	64.32±4.99	19.73±3.82	30.32±1.36	18.01±2.62	
B Thal intermedia	2	1	3 (0.3%)	46.70±1.01	33.73±4.31	7±2.23	2.15±0.74	4.87±2.25	13.47±5.13	61.9±2.76	20.83±3.65	31.63±2.29	35.23±6.21	
B Thal Major	5	1	6 (0.6 %)	89.85±7.27	4.75±0.82	1.63±0.4	2.32±0.77	5.73±1.92	18.55±6.09	79.87±2.42	24.67±1.45	30.87±2.23	35.35±4.96	
Sickle cell Trait	0	4	4 (0.4%)	1.83±1.7	51.78±2.91	2.43±0.85	4.84±0.6	11.95±2.44	39.55±7.7	76.11±7.34	23.17±2.38	30.15±0.73	16.70±2.31	**33.83± 3.59**
HbS/ Beta	1	0	1 (0.1%)	2.8	4.3	4.6	3.41	8.4	25	73.3	24.6	33.6	21.5	71.90
Hb E Trait	5	8	13 (1.31%)	0.88±0.14	61.25±4.57	32.05±6.24	5±0.79	11.01±2.65	35.65±7.11	70.64±4.8	22.52±4.1	30.68±2.21	16.82±2.92	
Hb E Homo	4	3	7 (0.7%)	3.44±2.99	6.56±0.88	90.09±5.08	4.86±0.88	8.81±2.12	31.36±6.09	64.46±3.17	26.86±23.54	29.40±4.86	20.97±4.77	
HbE/ Beta	1	1	2 (0.2%)	18.55±9.83	8±0.28	61.95±9.97	4.0±2.13	7.05±3.18	25.4±14.28	47.95±19.02	18.1±1.7	28.80±3.68	30.95±7.28	
Hb D	0	3	3 (0.3%)	0.83±0.06	52.63±3.88	2.40±0.26	4.70±0.31	11.07±2.32	30.47±4.53	71.67±6.52	23.4±3.53	32.53±2.56	14.83±1.88	33.10± 1.14
Hb H	1	0	1 (0.1%)	HbA1a 17.6%	55.20	0.40	2.72	5.50	32.40	82.40	20.20	24.60	29.60	Unknown 22.3%
Delta Thal	1	2	3 (0.3%)	0.8±0	84.9±2.82	0.67±0.12	4.75±0.18	8.57±0.21	32.13±2.68	67.7±7.02	18±0.26	26.77±2.49	21.27±1.38	
Borderline A2	5	4	9 (0.9%)	1.29±1.06	76.64±12.39	3.38±.09	5.25±0.49	12.33±2.74	33.41±5.80	70.76±5.67	23.23±5.34	31.51±1.59	16.20±3	
Hb Lepore	0	1	1 (0.1%)	0.8	83.7	12.9	4.68	9.3	33.7	72	19.9	27.6	25.9	

In the present study, 274 cases (27.41%) showed abnormal Hb fractions in HPLC analysis. The major abnormalities were of high HbA2 level of value > 3.5% was taken for diagnosis of Beta thalassemia trait (Ryan et al., 2010). A cut-off of HbA2 3.5% or above has been

Table 2: Distribution of normal chromatograms and chromatograms with Hb variant among different age groups.

Hemoglobin variants	Possible Alpha/ IDA	β thal trait	β thal inter-mediate	β thal Major	sickle cell Trait	HbS/ Beta	Hb E Trait	Hb E Homo	Hb E/ Beta	Hb D Trait	Hb H	Delta	Hb Lepore	Border-HbA2	Total
No of Cases	722	221	3	6	4	1	13	7	2	3	1	3	1	9	996
<1 year	25	1	1	3	0	0	0	0	0	0	0	0	0	0	30
Children (<12)	112	15	2	3	1	0	0	0	1	1	0	0	0	4	139
Adolescent (12-19)	56	14	0	0	0	0	1	1	0	0	0	0	0	0	72
Adult (20 - 40)	343	124	0	0	3	1	5	5	1	1	1	1	1	3	489
Post Adult (41 - 60)	160	48	0	0	0	0	6	1	0	1	0	1	0	2	219
Old Age (>60)	26	19	0	0	0	0	1	0	0	0	0	1	0	0	47
Chromatogram Pattern	Normal	Chromatogram with Hemoglobin variant											Normal		

cases and not in normocytic and normochromic, HbA2 level of value > 3.5 was taken for diagnosis of Beta thalassemia trait. 722 cases showed normal chromatogram pattern with normal and decreased HbA2 values (Table 2).Without increase in HbA2 levels (HbA2 1.0-3.4) these hematological alternations may be due to alpha thalassaemia, silent beta thalassemia, iron deficiency or occasionally chronic disease anemia (Weatherall and Clegg, 1981; Bunn, 1986; Borges *et al.*, 2001 and Colah *et al.*, 2007).

Beta Thalassemia

Among 996 microcytic hypochromic anemia cases, 221 cases (22.19%) showed HbA2 values ranging from 3.6-8.4 %, in which 11 cases showed hemoglobin ranging 3.0 -7.8 g/dl and 210 cases showed Hb ranging from 8.1-15.9 g/dl. Among 221 cases, 6 cases showed HbA2 values ranging from 3.6-3.9 % and Hb ranging from 9.6-15.9 g/dl this indicates heterozygosity for beta thalassemia trait. The present study showed similarity with the report of Rangan *et al.* (2011) in North India. Three cases of beta thalassemia intermedia and 6 cases of beta thalassemia major were presented with HbA2 4.6 – 9.0 & 1.0 -2.2 respectively. Hb fractions on HPLC and RBC indices in various hemoglobinopathies are shown in Table-1.

In this study, among microcytic hypochromic anemia, 221 cases (22.19%) showed beta thalassemia trait. The cutoff of HbA2 values in various studies have been presented in Table-3.The prevalence rate was higher when compared to other studies conducted in various parts of the India, 8.9 % reported in North India (Sachdev *et al.*, 2014) with 3.9% as HbA2 cutoff, 10.49% & 11.55% in western India (Philip *et al.*, 2013 and Shrivastav *et al.*, 2014) with 3.9% as HbA2 cutoff, 9.62% in eastern India (Chaudhury *et al.*, 2013) with 3.5% as HbA2 cutoff and 23.2% in south India, out of 543 abnormal chromatograms (Chandrashekar and Soni (2011) with reference range of HbA2 are 1.8-4.0%, ie >4.0%) respectively. It may be recalled that for diagnosis of beta thalassemia based on their studies different authors have established and followed different cutoff values for HbA2 (Table-3) which include borderline HbA2 values based on their molecular studies. As discussed earlier, in the present study >3.5 % is set as a HbA2 cutoff for diagnosis of beta thalassemia. However, borderline cases should confirm with molecular analysis.

Table 3 : HbA2 Cutoff % for Beta thalassemia among various studies.

Screening Criteria	MCHC	MCHC	MCHC	General Population	MCHC	MCHC	General Population	Microcytic	General Population	MCHC	MCHC
Author	Present study	Colah *et. al.*	Ryan *et. al.*	Sachdev *et. al.*	Chandra-shekhar & Soni	Rangan *et. al.*	Chaudhury *et. al.*	Denic *et. al.*	Shrivas-tava *et. al.*	Philip *et. al.*	Pant *et. al.*
Year		2007	2010	2010	2011	2011	2013	2013	2013	2014	2014
HbA2 %	>3.5	>3.5	>3.5	>3.9	>4.0	>3.5	>3.5	>3.5	>3.9	>3.9	>3.5

Three cases of β thalassemia intermedia (0.3%) have showed severe anemia with (Hb averaging 4.8 g/dl), raised HbF levels averaging 46.7% and increased HbA2 averaging 7.0 %. Six cases of β thalassemia major (0.6 %) of severe anemia, (Hb averaging 5.73%), HbF levels raised averaging 89.85% and HbA0 averaged 4.75 % and decreased HbA2 averaging 1.63 %. In the light of present investigation, it is inferred that microcytic hypochromic anemia serves as an important marker to detect the thalassemia carrier and to offer genetic counselling and prenatal screening of the spouse to prevent the birth of children with beta thalassemia major in future.

Borderline HbA2

In this study, among 996 microcytic hypochromic anemia cases, nine cases were observed with borderline HbA2 values between 3.2 % - 3.5 %, Hb ranging from 9.7 to 18.3 and RBC count ranging from 4.22 to 6.28 and RDW CV mean of 16.2%. Cases with HbA2 between 3.2% and 3.5% were at the risk of false negative diagnosis of beta thalassaemia trait (Denic et al., 2013). In addition, co-inheritance of alpha thalassemia mutations with beta thalassemia mutation may lower the HbA2 level. (Harteveld and Higgs, 2010 and Denic *et al.*, 2013) (Table 3). In order to prevent the risk of false negative diagnosis of beta thalassemia, DNA analysis is warranted to rule out both alpha thalassemia and beta thalassemia trait.

Possible Alpha Thalassemia/Iron Deficiency

In the absence of Hb variant, β or δβ thalassemia heterozygosity, α thalassemia cases are considered with MCH <25pg (Ryan *et al.*, 2010). Alpha thalassemia have a variable degree of anemia, reduced MCV, reduced MCH, and normal or slightly reduced level of HbA2 (Harteveld and Higgs, 2010). In this study, among 996 samples, 274 cases showed Hb variants (includes beta and other variants) and 722 cases showed no hemoglobin variants. However, out of 722 cases without any abnormal Hb variant 247 cases with Hb < 8.0 g/dl showed low HbA2 (1.1-2.2%) and 35 cases with normal HbA2 (2.2-3.0%) with RDW averaging 22.06 % were observed, probably due to anemia or alpha thalassemia. (Table 4). It may be stated that severe iron deficiency anemia (Hb<8.0 g/dl) can reduce Hb A2 (Ryan *et al.*, 2010).

In the present investigation, among 722 cases, 440 cases showed Hb>8.0 g/dl. Out of these 440 cases, 247 cases have low HbA2 (1.0-2.2) and 193 cases have normal HbA2 (2.2-3.1) with RDW averaging about 18.16% which is probably due to deletional or Non-deletional alpha thalassaemia (Table-4). In normal chromatograms with microcytic and hypochromic condition alpha thalassemia trait may occur, which does not exclude alpha thalassemia trait (Bain, 2006). A co-existent iron deficiency anemia or alpha thalassemia trait needs to be ruled out with iron, ferritin levels and DNA analysis (Ronald, 2006, Ryan *et al.*, 2010, Denic *et al.*, 2013, Philip *et al.*, 2013).

In HbH disease, there is a deficiency of alpha globin chains resulting in an α/β globin chain ratio of 0.2 to 0.7 (Chui et al., 2003). HbH are intracellular precipitates of β4 tetramers and forms golf ball like inclusions when it is stained with brilliant cresyl blue. (Ryan *et al.*, 2010). However, in this present study, one case of HbH has numerous golf ball like inclusions which was confirmed by incubating with Brilliant Cresyl Blue. Chromatogram showed low HbA2 - 0.4 % with elevated HbA1a (17.6%) and unknown peak (22.3%)

Table 4 : Hemoglobin, HbA2 and RDW among 722 Normal chromatogram cases with microcytic hypochromic anemia cases.

Hb < 8.0 g/dl with RDW averaging 22.06 % (282 Cases)		Hb≥ 8.0 g/dl with RDW averaging 18.16 % (440 Cases)	
HbA2 (1.1-2.2%) 247 Cases	HbA2 (2.2-3.0%) 35 Cases	HbA2 (1.0-2.2%) 247 Cases	HbA2 (2.2-3.1%) 193 Cases
Possible for anemia or alpha thalassemia		Possible for deletional or non-deletional alpha thalassemia	

with retention time of 0.16 and 0.38 minutes respectively. RBC indices showed low Hb of 5.5% presented with mild microcytic and severe hypochromic anemia.

HbE and Mixed Variants

In beta globin gene, glutamic acid is substituted for lysine at 26th position which causes Hb E variant hemoglobin and elutes in HbA2 window on HPLC (Sachdev *et al.*, 2010 and Shrivastav *et al.*, 2013). It is the most common Hb variant in Southeast Asia and second most prevalent in worldwide (Vichinsky, 2007 and Shrivastav *et al.*, 2013).

In this study, among 996 cases, 22 cases showed HbE variant, which include 13 cases (1.31%) heterozygous, 7 cases (0.7%) homozygous and 2 cases of (0.2%) HbE β-thalassemia. In the present study, the percentage of HbE is 32.05% in HbE Trait, 90.09 % in HbEE with mild elevation of HbF averaged about 3.44% and 61.95% with HbF about 18.5% in HbE β-thalassemia.

In HbE heterozygous about 30% of HbE has been reported by Vichinsky (2007) and Sachdev et al. (2010) where as 29.3% was reported by Philip *et.al.*, (2013). About 90% of HbE in homozygous and 40-60 % of HbE in HbE β-thalassemia with increased HbF has been reported by Vichinsky (2007) and Philip *et al.*, (2013) which is similar to the present study.

Sickle Cell Disease and Mixed Variants

In beta globin gene, valine is substituted for glutamic acid at 6th position (GAG→GTG) which causes HbS variant hemoglobin and tends to elute in S window on HPLC. Among 996 cases, 4 cases of HbS trait and 1 case of HbS β-thalassemia were found. In sickle cell heterozygous and homozygous cases blood smear showed normocytic and normochromic blood cells or reduced when associated with alpha thalassaemia trait (Bain, 2006). However, the present investigation was carried out with microcytic hypochromic anaemia cases, therefore prevalence rate of Hb S disorders was less when compared to other studies. HbS accounts for 30.9 % in HbS trait, 65.6% in HbS β-thalassemia (Chandrashekar and Soni, 2011 in Chennai). In this study HbS is found to be 33.83% in HbS trait and 71.9 % in HbS β-thalassemia.

HbD Trait

HbD Punjab is the fourth most common hemoglobin variant world wide. In beta globin gene, glutamine is substituted for glutamic acid at 121st position (GAA→CAA) which causes HbD Punjab variant and tends to elute in an unknown window with retention time of approximately 3.8 minutes on HPLC (Bain, 2006).Besides there are 7 other types of HbD present, caused by different point mutations in HBB. This mutation originated in central region of Asia, is prevalent in Punjab of India (Torres, 2014). In the present study, three cases of HbD were observed with abnormal hemoglobin about 33.1 % of total hemoglobin at retention time of approximately 3.85 min and HbA2 ranging between 2.2 to 2.7 % with normal HbF.

Delta Thalassemia

In hemoglobin about 3% of adult hemoglobin is made of alpha and delta chains. A mutation in delta chains affects the synthesis of HbA2 which causes delta thalassemia to be of no clinical significance (Bain, 2006). However delta thalassemia when co-inherited with beta thalassemia results in normal HbA2 level and misleads in beta thalassemia identification (Moi, 1992). In this study, three cases of delta thalassemia with microcytic hypochromic anemia and low HbA2 of 0.67% were observed. Molecular analysis is recommended for confirmation.

Minor Hemoglobin Variants.

Several other rare types of thalassemic disorders and haemoglobin variants have been sporadically reported from various parts of India (Chandrashekar and Soni, 2011 and Philip et al., 2013). These include hereditary persistence of fetal haemoglobin, Hb Lepore, Hb Q etc. Majority of these cases are rare and of not much clinical consequences. In this study, one case of Hb Lepore trait with HbA2 of 12.9 % and is presented with microcytosis and hypochromia. Variant hemoglobins are clinically significant, but many are clinically silent. However coinheritance of two variants may lead to severe consequences.

CONCLUSION

In conclusion, among 996 cases, about 27.3% of hemoglobinopathies and thalassemia were noted among MCHC anemia, which is comparable and higher than the studies of Philip *et al.* (2013). Generally, microcytosis and hypochromia are often interpreted as indicators for iron deficiency. In the light of present investigation it is inferred that high prevalence of Hemoglobinopathies and thalassemia are presented with microcytic hypochromic anemia. Moreover microcytic hypochromic condition which serves as an excellent diagnostic marker to detect Hemoglobinopathies. Therefore genetic studies are recommended for confirmation of alpha thalassemia and other borderline cases of beta thalassemia.

In this present study, it has been revealed that hemoglobinopathies and thalassemia are major health problems among the people of Chennai. The effective preventive approaches to thalassemia must be adopted by the Government with carrier screening programmes. The data summarized in this study, confirm that screening and genetic counselling for hemoglobin disorder in microcytic hypochromic anemia cases ensure a recommended baseline for diagnosis.

ACKNOWLEDGEMENTS

Authors are thankful to the HOD of Zoology and the Principal of Pachaiyappa's college for their encouragement to complete the research project. The authors are extremely grateful to Dr. Sp. Ganesan, Director of Hitech Diagnostic Centre, Chennai for providing technical support and suggestions.

REFERENCE

Bain, B J., 2004. A Beginner's Guide to Blood Cells, 2nd edition.

Bain, B J., 2006. Haemoglobinopathy Diagnosis Blackwell, Oxford, UK, 2nd edition, pg 1-314.

Balgir, R. S., 1999. Control and prevention of genetic load of hemoglobinopathies in India. *Nat Med J India*, **12**, 234-238.

Balgir, R. S., 2000. The burden of haemoglobinopathies in India and the challenges ahead. *Curr sci*, **79(11)**, 1536-47.

Balgir, R. S., 2005. Spectrum of hemoglobinopathies in the state of Orissa, India: a ten years cohort study. *JAPI*, **53**, 1021-1026.

Borges, E., Kimura, E. M., Gervásio, S. A., Costa, F. F., & Sonati, M. F., 2001. High prevalence of alpha-thalassemia among individuals with microcytosis and hypochromia without anemia. *Brazilian Journal of Medical and Biological Research*, **34(6)**, 759-762.

Chandrashekar, V., & Soni, M., 2011. Hemoglobin disorders in south India. ISRN hematology.

Chaudhury, S. R., Roy, H., Bhattacharyya, N. K., Mondal, P. K., Biswas, P. K., & Roy, S., 2013. Spectrum of hemoglobin variants in Eastern Indian population; a study of 14,145 cases. *Al Ameen J Med Sci*, **6**, 243-8.

Chui, D. H., Fucharoen, S., & Chan, V., 2003. Hemoglobin H disease: not necessarily a benign disorder. *Blood*, **101(3)**, 791-800.

Colah, R. B., Surve, R., Sawant, P., D'Souza, E., Italia, K., Phanasgaonkar, S., & Gorakshakar, A. C., 2007. HPLC studies in hemoglobinopathies.*The Indian Journal of Pediatrics*, **74(7)**, 657-662.

de Souza Torres, L., Okumura, J. V., da Silva, D. G. H., & Bonini-Domingos, C. R., 2015. Hemoglobin D-Punjab: origin, distribution and laboratory diagnosis. *Revista brasileira de hematologia e hemoterapia*, **37(2)**, 120-126.

Denic, S., Agarwal, M. M., Al Dabbagh, B., El Essa, A., Takala, M., Showqi, S., & Yassin, J., 2013. Hemoglobin A 2 lowered by iron deficiency and α-thalassemia: should screening recommendation for β-thalassemia change?.ISRN hematology.

Fucharoen, S., & Winichagoon, P., 2011. Haemoglobinopathies in southeast Asia. *The Indian journal of medical research*, **134(4)**, 498.

Giambona, A., Passarello, C., Vinciguerra, M., Muli, R. L., Teresi, P., Anzà, M., & Maggio, A., 2008. Significance of borderline hemoglobin A2 values in an Italian population with a high prevalence of β-thalassemia. *haematologica*, **93(9)**, 1380-1384.

Guideline- The Laboratory Diagnosis of Hemoglobinopathies., 1998. *British Journal of Hematology*. **101**, 783-792

Harteveld, C. L., & Higgs, D. R., 2010. Review α-thalassaemia. *Orphanet J Rare Dis*, **5**, 13.

Higgs, D. R., 2013. The molecular basis of α-thalassemia. Cold Spring Harbor perspectives in medicine, *3(1)*, a011718.

Kiss, T. L., Ali, M. A., Levine, M., & Lafferty, J. D., 2000. An algorithm to aid in the investigation of thalassemia trait in multicultural populations. Archives of pathology & laboratory medicine, **124(9)**, 1320.

Kumar, R., Gupta, S., Jindal, A., Kakkar, S., & Kaur, A., 2015. Screening of β-thalassemia trait and other hemoglobinopathies among blood donors in Punjab. *International Journal of Medicine and Public Health*, 5(1), 106.

Lupski, J. R., & Stankiewicz, P., 2005. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genet*, **1(6)**, e49.

Modell, B., & Darlison, M., 2008. Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization*, **86(6)**, 480-487.

Moi, P., Loudianos, G., Lavinha, J., Murru, S., Cossu, P., Casu, R., & Pirastu, M., 1992. Delta-thalassemia due to a mutation in an erythroid-specific binding protein sequence 3'to the delta-globin gene. *Blood*, **79(2)**, 512-516.

Nadeem,R., Ahmed,N., & Bashir, S, 2012. Frequency of iron deficiency anemia and beta thalassaemia trait at hematology department of children hospital, Lahore.*Biomedica*, **28**, 67.

Pant, L., Kalita, D., Singh, S., Kudesia, M., Mendiratta, S., Mittal, M., & Mathur, A., 2014. Detection of Abnormal Hemoglobin Variants by HPLC Method: Common Problems with Suggested Solutions. International Scholarly Research Notices.

Philip, J., Sarkar, R. S., & Kushwaha, N., 2013. Microcytic hypochromic anemia: Should high performance liquid chromatography be used routinely for screening anemic and antenatal patients?. *Indian Journal of Pathology and Microbiology*, **56(2)**, 109.

Rangan, A., Sharma, P., Dadu, T., Saxena, R., Verma, I. C., & Bhargava, M., 2011. β-Thalassemia mutations in subjects with borderline HbA2 values: a pilot study in North India. *Clinical Chemistry and Laboratory Medicine*, **49(12)**, 2069-2072.

Ryan, K., Bain, BJ., Worthington, D., James, J., Plews, D., Mason, A., Roper, D., Rees, D.C., Salle, B., Streetly, A., 2010. Significant haemoglobinopathies: Guidelines for screening and diagnosis. *Br J Haematol* ;**149**:35-49

Sachdev, R., Dam, A R., and Tyagi, G., 2010 "Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases," *Indian Journal of Pathology and Microbiology*, vol. **53(1)** pp. 57–62.

Shrivastav, A., Patel, U., Joshi, J. R., Kaur, A., & Agnihotri, A. S., 2013. Study of hemoglobinopathies and Hb variants in population of Western India using HPLC: A report of 7,000 cases. *Journal of Applied Hematology*, **4(3)**, 104-9.

Trent, R J A., 2006. Diagnosis of Hemoglobinopathies. *Clin Biochem Rev* Vol 27.

Urrechaga, E., Borque, L., & Escanero, J. F., 2011. The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and β-thalassemia screening. *American journal of clinical pathology*, **135(3)**, 374-379.

Vichinsky, E., 2007. Hemoglobin E syndromes. ASH Education Program Book, 2007(1), 79-83.

Weatherall, D. J., & Clegg, J. B., 2001. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Health Organization, 79(8), 704-712.

WHO, 2007. Management of Hemoglobin Disorders. Report of joint WHO-TIF meeting on the Management of Hemoglobin Disorders. Nicosia, Cyprus, 19-18, pp. 1–2.