Prevalence of HbA2´and its impact on diagnosis of β-thalassemia minor in Pakistan

Dr Bushra Moiz

Consultant Hematologist & Assistant Professor

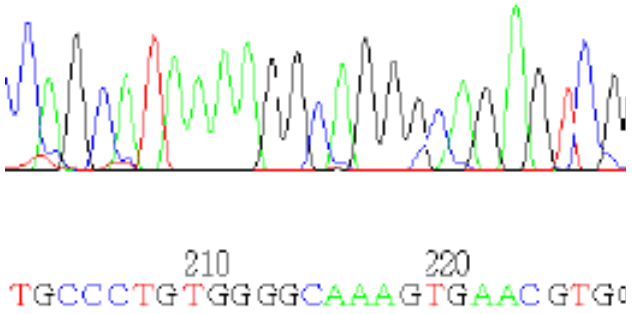
bushra.moiz@aku.edu

Introduction

- Hemoglobin (Hb) A2´, also called HbB2 or HbA2δ´, is globally the commonest δ chain variant of HbA2.

Annals of hematology. 2002 Jul;81(7):386-8.

- It was first discovered in 1958 in black community of Gulla James Island

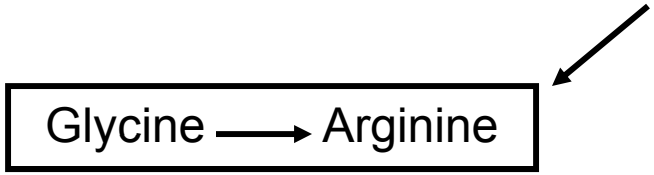


Introduction

HbA2´ is a clinically silent hemoglobinopathy that results from modification of δ globin gene (GGC >CGC) substituting glycine for arginine at codon 16

Hemoglobin. 2003 May;27(2):105-10.

Normal= GCCCTGTGGGGCAAAGTGAAC
Mutant = GCCCTGTGGCGCAAAGTGAAC



NCBI Reference Sequence: NM_000519.3

Introduction

The clinical significance of identification of HbA2´ is in the detection of co-existing β-thalassemia trait as in the presence of HbA2´ such individual might present with normal HbA2 levels resulting in under diagnosis of thalassemia minor

HbA2'+HbA2 >4.0% = beta thalassemia minor

Int J Lab Hematol. 2009 Jun;31(3):315-9.

Introduction

Diagnosis of HbA2´

- Alkaline electrophoresis
- Iso-electric focusing
- Micro-column chromatography
- HPLC

Clin Chem Acta. 2001 Nov;313(1-2):187-94.

Aims and objectives

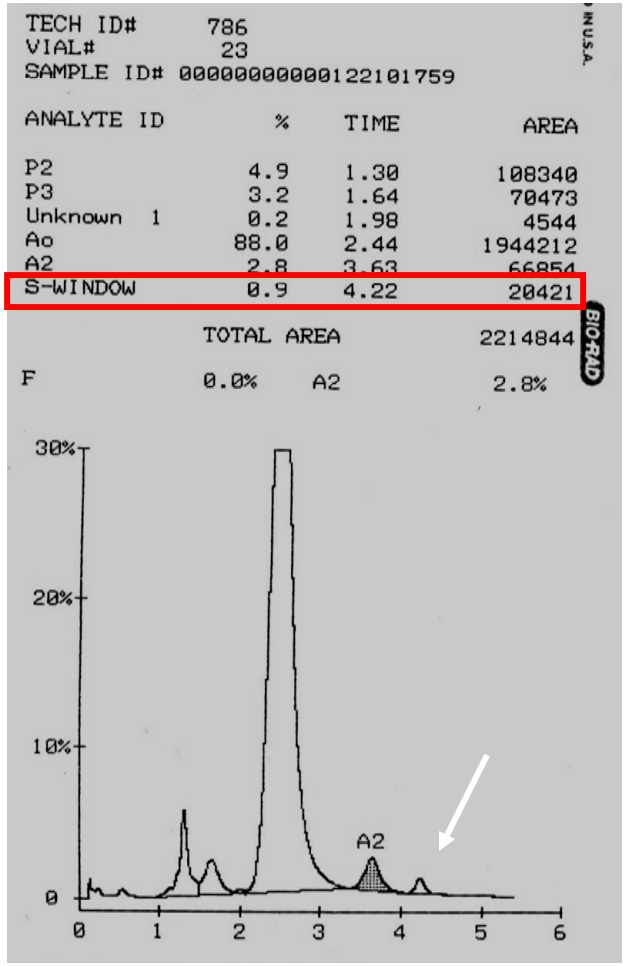
- The primary objective of the study was to evaluate the prevalence of HbA2´ and the true estimate of β-thalassemia carriers in Pakistani population.

- The secondary objective was to determine reference ranges for diagnosis of this variant by HPLC.

Material and Methods

- Year of interest: 2006
- Place of study: clinical lab, AKUH
- Type: Descriptive observational study
- Demographic data : IT
- Hematological parameters: Coulter®
- HPLC: Variant β-thalassemia short program (Bio-Rad Laboratories, Hercules, CA, USA
- Statistical analysis: SPSS version 16

Criteria for diagnosis of HbA2

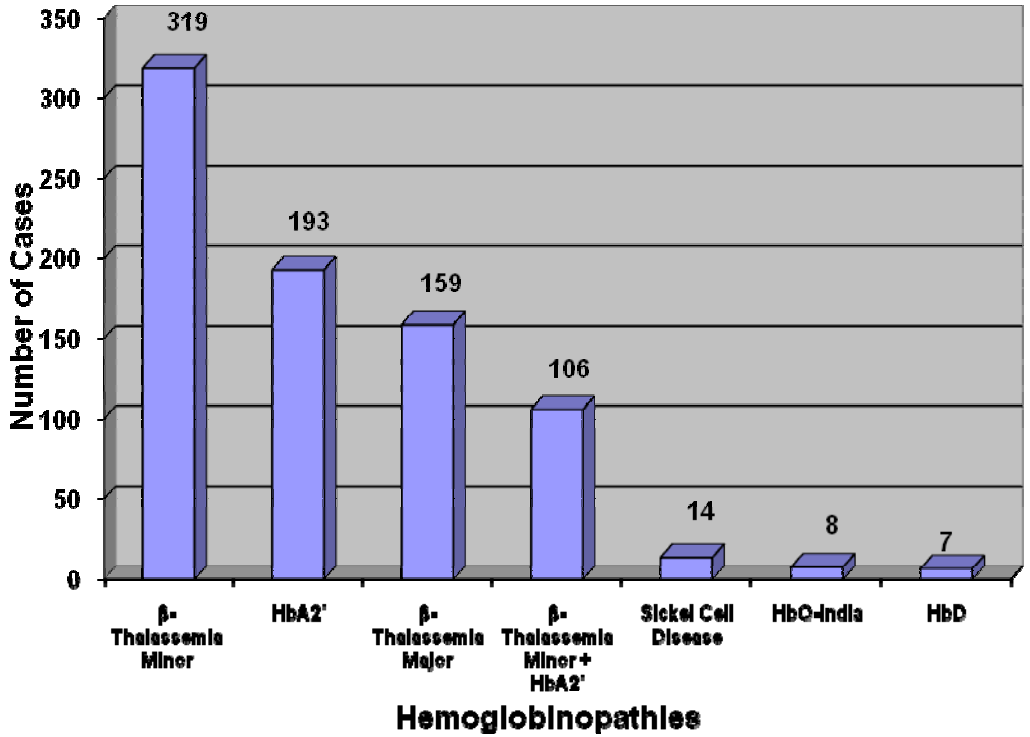


- S-window peaks (RT=4.5) of <4%
- Peaks of 1-2% .... HbA2´ trait
- HbA2´ >2% with HbA2 =0.... Homozygous HbA2´
- HbA2 + HbA2´ > 4.0% ....Beta thalassemia trait

Results

Various hemoglobinopathies seen in 2006 (n=10186)

- Total hemoglobinopathy=699(6.8%)



- ß thal trait=45.6%
- HbA2'=27.4%
- ß thal major=22.7%
- HbS=2%
- HbQ=1.1%
- HbD=1.0%

Demographic and lab data

- N=192 with S-window peaks
- Gender=90 males/79 females/23U
- Age mean±SD(range)=15.77 ±16.43 (0-76)
- Age<18 years= 87(45%)
- HbA2 (%)=3.30±2.05
- HbA2´(%)=0.92±0.47
- RT(min)=4.59 ± 0.05

HbA2’ levels

- HbA2´<1% = 123 (64.2%)
- HbA2´ 1-2% = 63 (32.6%)
- HbA2´ >2% = 6 (3.1%)

Analysis of 192 samples with HbA2´ and identification of 64 missed cases of β - thalassemia minor during 2006

HbA2´	Total Cases n (%)	Retention Time Range (min) Mean ± SD	HbA2* Range (%) Mean ± SD	Cases with HbA2 > 3.5% n (%)	Cases with HbA2+HbA2´>4.0% n (%)	Possibly missed β-thal n (%)
<1%	123(64.2)	4.33-4.62 4.59 ± 0.04	1.0-7.0 3.22 ± 1.41	28 (22.6)	48 (39.5)	20 (16.9)
1-2%	63 (32.6)	4.32-4.62 4.59 ± 0.05	1.3-6.3 3.07 ± 1.08	10 (15.9)	51 (81)	41 (65.0)
>2%	6 (3.1)	4.44-4.61 4.56 ± 0.06	2.3-5.2 3.67 ± 1.05	3 (50)	6 (100)	3 (50)
All cases	192(100)	4.32-4.62 4.58±0.05	1.0-7.0 3.18±1.29	41	105	64

* Difference in HbA2 levels in three groups is statistically not significant

Heterozygosity for HbA2´

Simple heterozygous= 87 (45.3%)

- HbA2´ % 0.74±0.21
- HbA2% 2.34±0.39
- HbA2+HbA2´% 3.08±0.37

Double heterozygous=105 (54.7%)

- HbA2´% 1.06±0.57
- HbA2% 4.09±2.49
- HbA2+HbA2´% 5.15±2.40

Homozygous=0

Hemoglobin levels

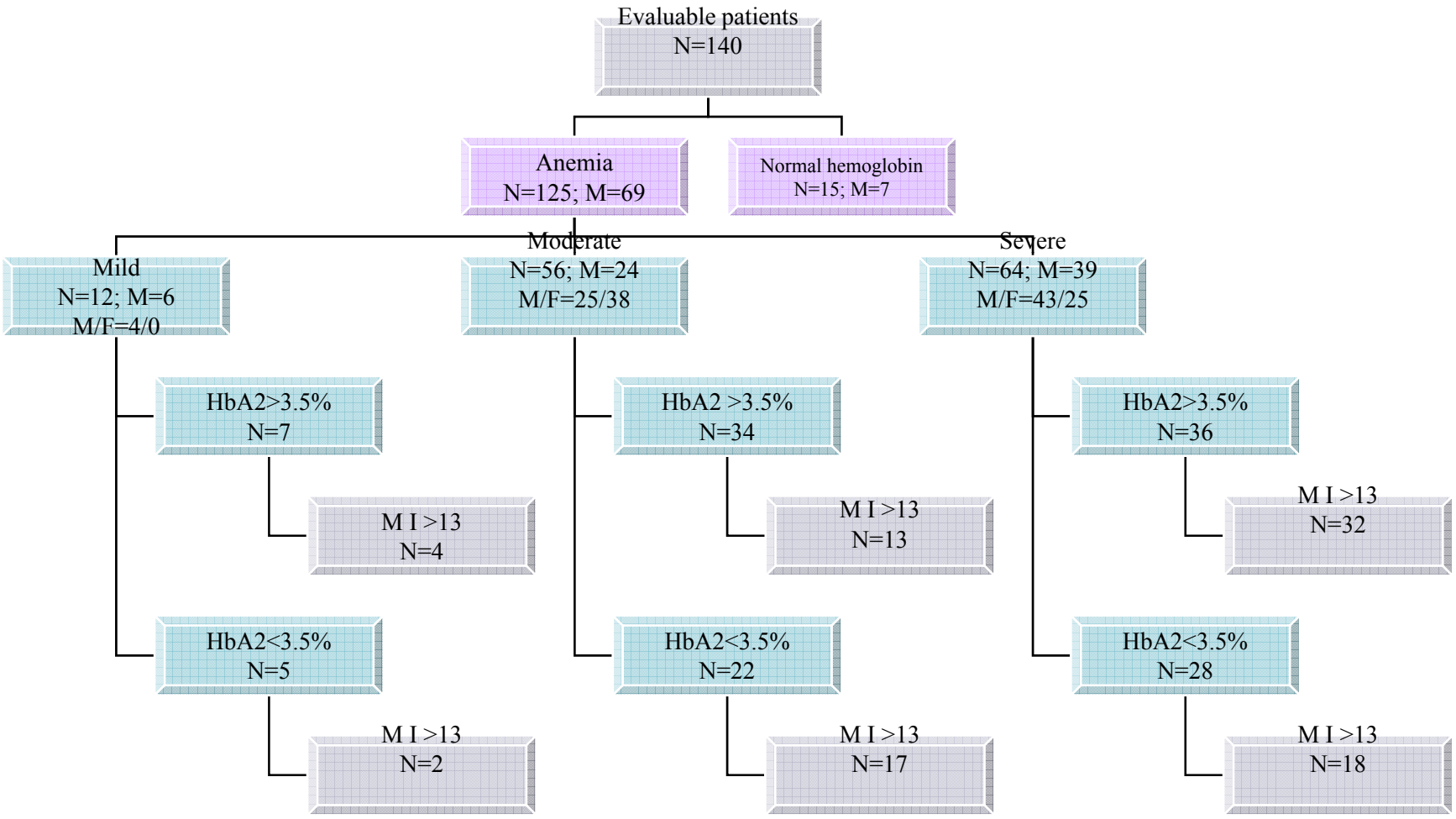
Simple heterozygous= 73

- Hb g/dl 8.46±2.87
- MCV fl 64.31±11.17
- MCH pg 19.77±5.05

Double heterozygous=92

- Hb g/dl 8.46±3.05
- MCV fl 66.42±13.01
- MCH pg 21.24±4.71

Cases with concomitant IDA



Discussion

- The prevalence of A2´ in our study was 1.89%, which is lower than that reported in other studies

Annals of hematology. 2002 Jul;81(7):386-8.

- A mean retention time of 4.59 ± 0.05 for HbA2´ cases was observed in the study which is also supported by others

American journal of clinical pathology. 2005 May;123(5):657-61.

Discussion

- Majority of our cases (64%) showed S-window peaks of less than 1%.

- Male presented significantly earlier than females with respect to age

Discussion

- This study showed HbA2 level in the range of 1.0- 2.9%for simple heterozygotes
- HbA2 <2% was defined

American journal of clinical pathology. 2005 May;123(5):657-61.

- In contrast, HbA2 level for double heterozygotes was 2.0-7.0%

Discussion

- Majority of our cases were compound heterozygotes for HbA2´ and β-thalassemia trait in three subgroups of HbA2.

Strengths and limitations

- First description of HbA2' and its impact on the diagnosis of beta thalassemia in Pakistan

- Molecular studies were not done
- CBC were not available for all cases
- Iron profile was not known

Conclusions

It appears that HbA2´ is widely prevalent in Pakistani population but since a considerable overlap was observed in HbA2 and HbA2´ levels in simple and double heterozygotes, we were unable to establish diagnostic criteria for these hemoglobinopathies.

Future trends

- Large prospective population based study is required
- HbA2' needs to be characterized at molecular level for confirmation

Practice points

- HbA2' elutes in S-window
- Technologists and pathologists should be aware of this
- HbA2' should be added to HbA2 to determine ß-thalassemia status


Thank you