Clinical Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

# Incidence of hemoglobinopathies and thalassemias in Northern Alberta. Establishment of reference intervals for HbF and HbA2

Karina Rodriguez-Capote *, Trefor N. Higgins

*DynaLIFE$_{DX}$ Diagnostic Laboratory Services, Edmonton, Alberta, Canada*
*Dept. Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada*

ARTICLE INFO

*Article history:*
Received 6 February 2015
Received in revised form 29 March 2015
Accepted 1 April 2015
Available online xxxx

*Keywords:*
Hemoglobinopathy screening
β-Thalassemia
α-Thalassemia
HbF reference interval
Hemoglobin $A_2$
$HbA_2$ reference interval
Hemoglobin analysis

ABSTRACT

**Objectives:** The aims of this study were to identify the incidence of hemoglobinopathies and thalassemias in Northern Alberta and calculate the reference intervals (RI) for hemoglobin (Hb) HbF and $HbA_2$.

**Methods:** A retrospective ad-hoc analysis of the structural Hb variants and thalassemias identified on patients who had a hemoglobinopathy/thalassemia investigation performed between February 1 to December 31, 2013. Results were extracted from the Laboratory Information System. Statistical analysis was performed using MedCalc® version 11.4.2.0 for Windows software.

**Results:** 6616 hemoglobinopathy/thalassemia investigations and HbS screens were physician requested and 602 Hb variants were fortuitously found during $HbA_{1c}$ analysis. 3438 were interpreted as "normal" and 532 were classified as iron deficient. 3306 individuals, with age ranging from 3 to 92 years were included in the RI calculation. $HbA_2$ RI was 2.3% to 3.4% and HbF 0.0% to 1.8%. 524 and 423 α and β thalassemia traits respectively were identified. Additionally ten δβ thalassemia traits and twelve cases of HbH disease were identified. Regarding hemoglobinopathies, 7% were classified as α-chain variants and 93% as β-chain variants with HbS (46%), HbE (16%), HbD Punjab (8%) and HbC (7%) traits being the most prevalent. We also documented 20 homozygous hemoglobinopathies and 36 compound/double heterozygous hemoglobinopathies.

**Conclusion:** A wide diversity of hemoglobinopathies is found in the Northern Alberta population, 80% of the hemoglobinopathies were found as a reflex to $HbA_{1c}$ testing. Reference intervals for HbF and $HbA_2$ were established.


## Introduction

Dyna*LIFE*$_{DX}$ is the sole laboratory performing hemoglobinopathy and thalassemia investigations for a catchment area of 2 million people in Northern Alberta, Canada. Hemoglobinopathy/thalassemia investigations came from physicians based on clinical presentation, immigration medicals, red cell exchange programs for sickle cell anemia patients and as a reflex test generated by the presence of a hemoglobin (Hb) variant noted during $HbA_{1c}$ analysis.

Diagnoses of these hemoglobin disorders are important for planning appropriate management and genetic counseling [1]. Additionally in Northern Alberta, screening for thalassemia and hemoglobinopathies is offered by the maternal care clinics if the patient and/or her partner are identified as belonging to an ethnic population whose members are at higher risk of being carriers as recommended by the clinical practice guideline from the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada and the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists [2]. Screening during pregnancy accounts for a high percentage of the requests for hemoglobinopathy and thalassemia investigations, since in Alberta neonatal hemoglobinopathy screening is not included in the Newborn Screening Program [3].

Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) is elevated in newborns, usually reaching adult levels by 12 months and it commonly increases up to 5% in normal pregnancy [4]. HbF measurement is useful in the diagnosis of β-globin gene disorders and in the prognosis of patients homozygous for HbS [5]. Some acquired conditions such as aplastic anemia and myeloproliferative disorders are also associated with mild increases of HbF. Furthermore, pharmacological stimulation of HbF is used to manage conditions such as sickle cell disease and β-thalassemia major [5,6]. Thus, a reliable monitoring of HbF concentration during the treatment and follow-up of these patients is required.

$HbA_2$ ($\alpha_2\delta_2$) is recommended as the primary test for the diagnosis of β-thalassemia trait in iron replete patients in the presence of microcytosis [1,7]. The slight difference between $HbA_2$ values in patients with and without β- thalassemia trait requires excellent precision at $HbA_2$ values

*Abbreviations:* CE-HPLC, cation-exchange high performance liquid chromatography; CI, confidence intervals; Hb, hemoglobin; HPFH, Hereditary Persistence of HbF; RI, reference interval.

* Corresponding author at: Dyna*LIFE*$_{Dx}$, #200, 10150-102 St, Edmonton, Alberta T5J 5E2, Canada. Fax: +1 780 454 2845.
*E-mail address:* karina.capote@dynalifedx.com (K. Rodriguez-Capote).

http://dx.doi.org/10.1016/j.clinbiochem.2015.04.001
0009-9120/

at the upper limit of the reference interval (RI). Thus quantification of $HbA_2$ has to be accurate.

A review of our data showed that 17% of HbF results reported in patients with a normal iron status and absence of a Hb variant exceeded the manufacturer's reference interval of <1.0%, therefore it was necessary to reassess the RI for HbF.

The aims of this study were to identify the incidence of hemoglobinopathies and thalassemias in Northern Alberta and calculate the reference intervals for HbF and $HbA_2$.

## Methods

This study is an ad hoc analysis of the structural Hb variants and thalassemias identified in the Northern Alberta, Canada on patients who had an investigation between February 1 to December 31, 2013. Pseudo-anonymised data containing only patient age, HbF and $HbA_2$ concentrations and interpretative results was extracted from the Laboratory Information System. This study complied with Dyna*LIFE*$_{DX}$ Institutional Ethical Board requirements.

In our laboratory, EDTA-anti-coagulated blood samples for hemoglobinopathy/thalassemia investigation and those with abnormal findings during $HbA_{1c}$ testing, are analyzed by a high resolution cation exchange high performance liquid chromatography (CE-HPLC) using the β thalassemia program on the Bio-Rad VARIANT II. The British Committee for Standards in Haematology recommends that a second methodology based on a different analytical principle be used to make a presumptive identification of the hemoglobin variant [8–11]. Following this principle any sample with an Hb variant fraction is further investigated by electrophoresis at alkaline and acid pH (Sebia Hydrasys Electrophoresis System). Detection of common α-thalassemia mutations and HbH disease are performed by GAP-PCR analysis (Calgary Laboratory Services). A complete blood count and ferritin tests are requested as part of the hemoglobinopathy/thalassemia investigation. The interpretative report is performed by the Clinical Biochemist, who integrates the patient results from the chromatogram, electropherograms, hematology indices and calculated Mentzer Index. Total imprecision (CV%) for HbF is 2.2% and 1.2% at levels of 2.17% and 9.54% respectively. For $HbA_2$ the total imprecision is 3.5% and 2.1% at levels of 2.8% and 5.7% respectively.

Data was imported to Excel (Microsoft Office 2010). Categorical variables were expressed as frequencies and percentages. 95% reference intervals with 90% confidence intervals (CI) were calculated using the non-parametric percentile method according to CLSI EP28-A3$_C$ [12]. Statistical analysis was performed using MedCalc® version 11.4.2.0 for Windows.

## Results

Of the 6616 thalassemia and hemoglobinopathy investigation requests 3312 were on patients older than 2 years and interpreted as "normal" by a Clinical Biochemist based on their hematology indices (hemoglobin >120 g/l, mean cell volume >80 fL), absence of a hemoglobin variant, replete iron status and calculated Mentzer Index (MCV divided by RBC > 14). These results were included in the calculation of the RIs for HbF and $HbA_2$. Both, $HbA_2$ and HbF failed the Shapiro–Wilk test for normal distribution (Table 1), even after logarithmic or Box–Cox transformation (data not shown). Consequently, their respective RIs were calculated by the percentile method as recommended by the CLSI C28-A3 guidelines [12]. Box-plots summarizing the data are presented in Fig. 1.

A summary of the hemoglobinopathy/thalassemia investigation main findings is presented in Fig. 2, the various hemoglobin variants diagnosed in this period are tabulated as follows: Table 2, α-chain variants; Table 3, β-chain variants and Table 4, Hb homozygous and double or compound heterozygous.

**Table 1**
95% reference intervals for $HbA_2$ and HbF.

| N = 3312 | HbF% | $HbA_2$% |
|---|---|---|
| LLRI (90% CI) | 0% | 2.3% (2.2–2.34) |
| ULRI (90% CI) | 1.8% (1.78–1.90) | 3.4% (3.4–3.5) |
| Coefficient of skewness | 1.97 (P < 0.0001) | −0.48 (P < 0.0001) |
| Coefficient of kurtosis | 6.27 (P < 0.0001) | 1.84 (P < 0.0001) |
| Shapiro–Wilk test | W = 0.83, reject normality (P < 0.0001) | W = 0.97, reject normality (P < 0.0001) |

CI, confidence interval; LL, lower limit; UL, upper limit; RI, reference interval.

## Discussion

Of the 6616 hemoglobinopathy/thalassemia investigations performed over a period of 11 months, 52% were considered "normal" and 8% were considered as iron deficient. The RIs obtained in our population [$HbA_2$ 2.3–3.4% and HbF 0.0–1.8%] are similar to those reported by other large reference laboratories such as ARUP Laboratories [$HbA_2$ 2.0–3.5% and HbF 0.0–2.1%] [14] and Mayo Medical Laboratories [$HbA_2$ 2.0–3.3% and HbF 0.0–0.9%] [15].

Hemoglobinopathies and thalassemias are frequent in the Mediterranean coastal region, India, Africa and in Southeast Asia, and therefore are commonly found in immigrants from these areas and their descendants [1,16]. Our laboratory serves a diverse multicultural urban population with the 2011 census results indicating that 22% of the Edmontonians were born outside Canada, from these 26% were from South Eastern Asia, 6% from the Mediterranean basin and 9% from Africa; accounting for around 14% of the total population [17,18].

Some structural Hb variant or thalassemia was found on 40% of the patients. 92% of the hemoglobin variants were β-chain variant traits with HbS (47%), HbE (16%), HbD (9%) and HbC (7%) traits being the most prevalent. Complex hemoglobinopathies such as compound or double heterozygosity due to co-inheritance of defects in α- and β-globin genes were detected in 36 patients, with the double heterozygote for sickle cell trait and α-thalassemia minor being the most prevalent.

For twenty-three α-chain variants and four β-chain variants, the HPLC pattern and electrophoresis at acid and alkaline pH were not distinctive enough to make a presumptive identification of the hemoglobin variant. Eighteen of these α-chain variants and two of the β-chain variants were fortuitously detected during $HbA_{1c}$ analysis and the

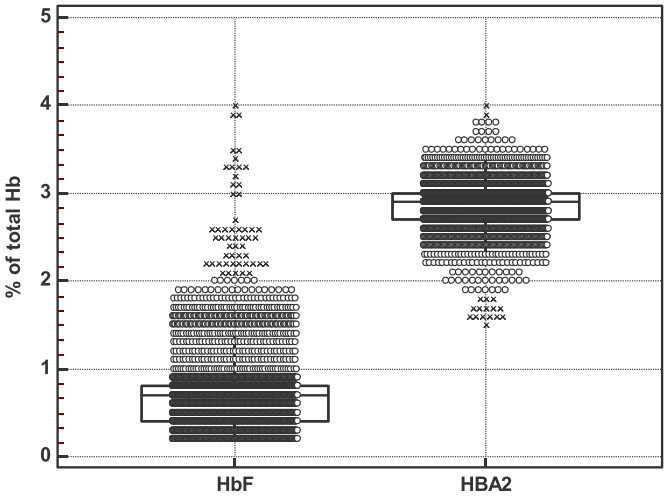


**Fig. 1.** Box-plot, the central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. Outlier values are displayed as X. Statistical analysis was performed using MedCalc® version 11.4.2.0 for Windows.

Please cite this article as: Rodriguez-Capote K, Higgins TN, Incidence of hemoglobinopathies and thalassemias in Northern Alberta. Establishment of reference intervals for HbF and HbA2, Clin Biochem (2015), http://dx.doi.org/10.1016/j.clinbiochem.2015.04.001