Annals of Hematology (2019) 98:2853–2855
https://doi.org/10.1007/s00277-019-03839-z

LETTER TO THE EDITOR

Hemoglobin Kirklareli [A_2 59(E7) His>Leu; HBA2:c.176A>T] in a Brazilian child with severe dyspnea and low O_2 saturation

G. A. Pedroso[1] · P. Fernandes[2] · S. E. D. C. Jorge[1] · P. H. Nascimento[1] · P. C. Lima[1] · M. R. P. Grigoleto[1] · D. M. Albuquerque[3] · M. N. N. Santos[1] · F. F. Costa[3] · A. A. D. C. Toro[2] · M. F. Sonati[1]

Received: 24 September 2019 / Accepted: 30 October 2019 / Published online: 11 November 2019

Dear Editor,

Hb Kirklareli was first described—and has to date only been reported—by Bissé et al. in 2017 [1] in a 23-year-old Turkish woman from the city of Kirklareli who presented with anemia associated with iron deficiency. Molecular analysis of the α globin genes and mass spectrometry of the patient's Hb peptides revealed the α1 H59L mutation (HBA1:c.176A>T] (according to Human Genome Variation Society-HGVS nomenclature). The patient's father was also a carrier of the mutation. A heavy smoker, he did not present with anemia but had abnormally high HbCO levels (around 16%). Structural and biophysical analyses demonstrated that the oxygenated form of Hb Kirklareli denatures very quickly under physiological conditions as the mutant α subunits undergo autoxidation and lose the heme more quickly than the native α subunits. The α H59L subunits, in contrast, have much greater affinity for CO than for O_2, preventing denaturation of the variant both in vitro and in vivo and explains the phenotypic differences between the patient and her father [1].

We detected Hb Kirklareli in an 11-year-old male Brazilian patient of Portuguese descent who had been admitted to the University Hospital, Unicamp. The patient presented with chronic obstructive respiratory insufficiency, and even after the obstruction had been resolved, O_2 saturation remained low (86–88%). He had a history of wheezing in the first year of life, and the episodes of dyspnea and bronchoconstriction restarted when he was 7 years old. The episodes were associated with physical effort and changes in the weather, and the patient was admitted to hospital on sporadic occasions. Although the child was already being treated for bronchial asthma (formoterol + budesonide and prophylactic azithromycin), no wheezes were heard on lung auscultation during exacerbations. Computed tomography of the thorax and ventilation/perfusion lung scan were unremarkable. The definitive diagnosis was atopic asthma.

After the parents had given their voluntary consent, peripheral blood samples were collected from the family. The hematologic and biochemical data for the patient and his parents are shown in Table 1. Only the father, who was diabetic and hypertensive, presented with anemia (normocytic and normochromic), while a slight reduction in iron was observed in both father and son; serum ferritin levels were within normal range.

Cation-exchange high-performance liquid chromatography (HPLC) (VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) failed to reveal any additional elution peak, but the fact that the baseline was distorted (Supplementary Material - Figure 1A) indicates that the hemolysate could usefully have been analyzed further. Reverse-phase HPLC (Waters Corporation, Milford, MA, USA) revealed the abnormal α chain (which accounted for 15% of the total globin), with a longer elution time than the normal α chain (Supplementary Material - Figure 1B). Tests to detect unstable Hb intra-erythrocyte bodies were negative [2].

Sequencing of the HBA1 and HBA2 genes (ABI PRISM® 3500, Applied Biosystems, Foster City, CA, USA) revealed the CAC→CTC substitution at codon 59 (HGVS nomenclature) of the HBA2 gene, which results in Hb Kirklareli [α2

Gisele Audrei Pedroso and Patricia Fernandes contributed equaly to the work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00277-019-03839-z) contains supplementary material, which is available to authorized users.

✉ M. F. Sonati
sonati@unicamp.br

[1] Hemoglobinopathies Laboratory, Department of Clinical Pathology, School of Medical Sciences, State University of Campinas – UNICAMP, Campinas, São Paulo, Brazil

[2] Department of Pediatrics, School of Medical Sciences, State University of Campinas – UNICAMP, Campinas, São Paulo, Brazil

[3] Center for Hematology and Hemotherapy, State University of Campinas – UNICAMP, Campinas, São Paulo, Brazil

Table 1 Hematologic and biochemical data for the patient and his parents

Subject	Patient	Father	Mother
Sex/age (years)	M/11	M/68	F/42
RBC ($\times 10^{12}$/L)	4.45	3.58	4.92
Hb (g/dL)	12.3	10.7	13.2
Ht (%)	35.3	32.0	39.5
MCV (fL)	79.3	89.4	80.3
MCH (pg)	27.6	29.9	26.8
RDW (%)	13.5	15.7	13.2
Reticulocytes (%)	1.54	3.88	0.85
Electrophoretic pattern	HbA_2 + HbA	HbA_2 + HbA	HbA_2 + HbA
Hb A2 (%)	0.8[a]	0.7[a]	2.8
Hb F (%)	0.1	0.2	0.1
Serum iron (g/dL)	66.0	62.0	88.0
Serum ferritin (ng/mL)	63.38	134.80	76.10
Haptoglobin (mg/dL)	73.9	160.0	154.0
Total bilirubin (mg/dL)	0.32	0.44	0.34
LDH (U/L)	195.0	135.0	148.0
Genotype	$\alpha^{Kirklareli}\alpha/\alpha\alpha$	$\alpha^{Kirklareli}\alpha/\alpha\alpha$	$\alpha\alpha/\text{-}\alpha^{3.7}$

[a] Percentage of Hb A2 (measurement accuracy potentially impaired by co-elution with variant Hb)

Reference values:

M, men/W, woman

Red blood cell (RBC): M = 4.5–6.1/W = 4.2–5.4/6–12 years = 4.0–5.2

Hemoglobin (Hb): M = 14.0–18.0/W = 12.0–16.0/6–12 years = 11.5–15.5

Mean corpuscular volume (MCV): M/W = 80–99/6–12 years = 77–95

Mean corpuscular hemoglobin (MCH): M/W = 27–32 6–12 years = 25–33

RDW: M/W = 10 to 15%

Reticulocytes: < 2.5%

Hb A2: 1.6–4.0%

Hb F (>6 months): < 2.0%

Serum iron: M = 70–180/W = 60–180

Serum ferritin: M = 30–400/W = 13–150

Haptoglobin: M/W = 30–230

Total bilirubin: M/W = 0.3–1.2

Lactate dehydrogenase (LDH): M/W = 140–271

59(E7) His>Leu; HBA2:c.176A>T], in the child and father, in a heterozygous state (Supplementary Material - Figure 1C). The mutation was confirmed by reverse strand sequencing. The most common forms of α-thalassemia (α-thal) (deletional and non-deletional) were excluded in the father and child [3, 4], but the mother is heterozygous for the $\text{-}\alpha^{3.7}$deletion ($\text{-}\alpha^{3.7}/\alpha\alpha$).

Functional characterization of the variant, which was performed with total hemolysate from the patient's father, who was also a carrier of the variant but was not taking any medication for asthma, was determined from the p50 on the Hb-O_2 saturation curve (pH levels between 6.5 and 8.5) using tonometry and spectrophotometry [5] (Supplementary Material - Figure 1D). Oxygen equilibrium studies indicated that the stripped lysate containing Hb Kirklareli had a lower O_2 affinity than the control at all pH levels (around 6 times less in pH level ≥ 8.0 and around 10 times less in a more acidic medium, i.e., pH level ≤ 7). The affinity of the lysate was also tested in the presence of the allosteric effector inositol hexaphosphate (IHP), a 2,3-BPG mimetic. Under these conditions, the affinity of the lysate containing Hb Kirklareli was similar to that of the control sample. Heme-heme cooperativity, determined by the Hill coefficient (n), resulted in similar n values to the stripped Hb A [$n_{\text{stripped-HbA(pH7.5)}} = 1.32$; $n_{\text{stripped-HbKirklareli(pH7.5)}} = 1.37$] and slightly decreased under the interference of IHP [$n_{\text{stripped-HbA+IHP(pH7.5)}} = 2.1$; $n_{\text{stripped-HbKirklareli+IHP(pH7.5)}} = 1.66$], indicating that the $Hb_{Kirklareli}$-O_2 ligation remains cooperative despite the significant reduction in O_2 affinity.

To our knowledge, this is the second reported case of Hb Kirklareli. The Brazilian patient did not present with anemia, but his respiratory condition may have been aggravated by the presence of the variant and reduced O_2 saturation even when he is not having an asthma attack. His father, who is diabetic and hypertensive, has moderate normocytic and normochromic anemia with a slightly increased reticulocyte count (3.9%) and plasma concentrations of hemolysis markers (haptoglobin, bilirubin, and LDH) within normal ranges.

He reported having given up smoking when he became diabetic; before that, however, he did not have anemia.

Stabilization of the protein by bonding with CO may explain the association between the abnormal Hb and the presence or otherwise of anemia in carriers of this variant. Our 11-year-old patient, however, neither smokes nor has anemia (never had), which suggests that other factors may also be involved in the structural stabilization of the Hb Kirklareli.

Funding information This study was supported financially by the State of São Paulo Research Foundation (FAPESP) (grant no. 2014/00984-3; fellowship no. 2015/13710-1), the National Council for Scientific and Technological Development (CNPq), the Agency for the Support and Evaluation of Graduate Education (CAPES), and the Fund for the Support of Teaching, Research and Extension (FAEPEX) of UNICAMP (Brazil).

Compliance with ethical standards

All procedures followed complied with the ethical standards of the UNICAMP committee on human experimentation and the Helsinki Declaration of 1975, as revised in 2008. The child's parents gave their written informed consent for this study.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Bissé E, Schaeffer-Reiss C, Van Dorsselaer A, Alayi TD, Epting T, Winkler K, Benitez Cardenas AS, Soman J, Birukou I, Samuel PP, Olson JS (2017) Hb Kirklareli (α H58L), a new variant associated with iron deficiency and increase CO binding. J Biol Chem 292: 2542–2555
2. Bain B, Bates I, Laffan M, Lewis S (2011) Dacie and Lewis practical haematology, 11th edn. Churchill Livingstone, New York
3. Tan AS, Quah TC, Low PS, Chong SS (2001) A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. Blood 98:250–251
4. Kattamis AC, Camaschella C, Sivera P, Surrey S, Fortina P (1996) Human alfa thalassemia syndromes: detection of molecular defects. Am J Hematol 53:81–91
5. Rossi-Fanelli A, Antonini E (1958) Studies on the oxygen and carbon monoxide equilibria of human myoglobin. Arch Biochem Biophys 77:478–492