



Hemoglobin (Hb) Val de Marne (Hb Footscray) in Brazil: the first case report

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ABSTRACT. Hemoglobin (Hb) variants involving alpha-chains are less common in the global population than Hb variants resulting from beta-chain alterations. Generally, alpha-chain Hb variants are caused by point mutations affecting alpha-1 and/or alpha-2 genes of the alpha-globin cluster (*HBA1* and *HBA2*). In Brazil, the most prevalent alpha-chain Hb variant is Hb Hasharon. In this study, we present the first case of an Hb Val de Marne variant in the Americas, specifically in Brazil.

Key words: Hemoglobin variants; Alpha-globin; HPLC

INTRODUCTION

Hemoglobin (Hb) variants are generally characterized by point mutations in globin genes, mainly in alpha- and beta-globin genes (*HBA* and *HBB*) modifying the formation of alpha- and beta-globin chains (Clarke and Higgins, 2000; Weatherall and Clegg, 2001). Owing to damaged tetrameric structure, the globins can form molecules with altered function; however, many mutations do not cause severe functional alterations, and the carriers are asymptomatic (Clarke and Higgins, 2000).

Of the 1218 Hb variants described, 587 result from mutations in the beta-globin cluster and 464 from mutations in the alpha-globin cluster. Regarding the point mutations (a change of a single nitrogenous base), 326 are related to beta-globin genes and 196 to alpha-globin genes (Globin Gene Server; <http://globin.cse.psu.edu/>). The most common Hb variants, both in Brazil and globally, are those that affect the beta gene (*HBB*), namely Hb S, Hb C, and Hb D (Weatherall and Clegg, 2001). The Hb variants resulting from changes in the alpha gene (*HBA*) are less frequent, and can be confused with other Hb variants during laboratory diagnosis. The most prevalent variant in Brazil is Hb Hasharon, followed by Hb Stanleyville II and Hb J-Rovigo (Kimura et al., 2015).

The Hb variant described in the present study, Hb Val de Marne (also known as Hb Footscray), is caused by replacement of the nitrogenous base cytosine by adenine at the 34th codon of the third exon of alpha-globin genes 1 or 2 (*HBA1* or *HBA2*), promoting exchange in the synthesized polypeptide chain of the amino acid serine (codon AGC) for arginine (codon AGA) at position 133 (HBA:p.Ser133Arg) (Wajcman et al., 1993; Owen and Hendy, 1994; Ma et al., 2004; Akbari and Hamid, 2012). Val de Marne is a rare Hb variant and has been described in only four families throughout the world, the first report being in 1993 in France (Wajcman et al., 1993), followed by Australia (Owen and Hendy, 1994), China (Ma et al., 2004), and Iran (Akbari and Hamid, 2012). The terms Hb Val de Marne (Hb VM) and Hb Footscray derive from the regions where this variant was first described.

We present here the first report of the Hb variant Val de Marne in the Brazilian population, and the first results of laboratory tests for Hb variants available in Brazil.

MATERIAL AND METHODS

Case

The subject of the case (L.T.M.) is a blood donor at the Regional Hemocenter of Maringá-PR, in the southern region of Brazil. L.T.M. is a 32-year-old Brazilian woman of Italian and Ukrainian ancestry. She lives a healthy life without any medical complaints and has no family history of anemia. As a preliminary analysis, the blood sample was subjected to hemoglobin electrophoresis at pH 8.6 and pH 6.2, a sickle test, and a complete blood count. However, the diagnosis for this sample was inconclusive.

Hemoglobin electrophoresis at pH 8.6 (Marengo-Rowe, 1965)

We used this qualitative technique for the detection of normal and abnormal Hb. The interpretation is based on different Hb electrical charges, which lead to different electrophoretic mobilities of the normal Hb. The running buffer used was Tris-EDTA-borate at pH 8.6

(Tris(hydroxymethyl)aminomethane) (Tris), 10.2 g/L (0.0842 M); ethylenediaminetetraacetic acid (EDTA), 0.6 g/L (0.002 M); boric acid, 3.2 g/L (0.0517 M)), and the hemolyzed sample was applied on cellulose acetate tapes as substrates.

Hemoglobin electrophoresis at pH 6.2 (Vella, 1968)

We used this qualitative technique to differentiate between Hb that migrate to similar positions such as Hb S and C under electrophoresis at alkaline pH. The running buffer used was sodium phosphate at pH 6.2 [Na_2HPO_4 , 2.02 g/L (0.0142 M) and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 7.66 g/L (0.0555 M)] and the hemolyzed sample was applied on agar-agar gel as substrate (1 g agar: 5 mL sodium phosphate buffer).

Polypeptide chains electrophoresis at pH 8.6 (Schneider, 1974)

We used this technique to identify normal and altered globin chains. The running buffer used was Tris-EDTA-borate at pH 8.6 (70 mL) with urea (36 g, 8562 M) and 2-mercaptoethanol ($\text{C}_2\text{H}_6\text{OS}$, 6.4 mL, 1.17 M). The hemolyzed sample was applied on cellulose acetate tapes as substrates.

Hemoglobin separation and quantification by high-performance liquid chromatography (HPLC)

Based on the ionic charge of each Hb fraction it is possible to identify the Hb profile and the percentage values of each fraction. We used VARIANT™ system (Bio-Rad Laboratories, Hercules, CA, USA) with an heterozygous beta thalassemia assay kit and Ultra2 Resolution system (Trinity Biotech, Wicklow, Ireland) with Genesys assay kit.

Hematological tests

The erythrocyte parameters were obtained using an Auto Hematology Analyzer, BC-300 PLUS (Mindray, China). Microscopic analysis of erythrocytes was performed using blood smears colored by May-Grünwald-Giemsa staining and visualized using a light microscope at 40X magnification.

RESULTS

The hematologic data did not reveal any alterations, except mild elevation in the red blood cell distribution width coefficient of variation (RDW-CV), indicating slight anisocytosis (Table 1). The data show that L.T.M. did not present anemia, erythrocytosis, or any relevant qualitative abnormalities in erythrocytes.

The electrophoretic migration pattern of the Hb VM observed at pH 8.6 occurred between the Hb A and Hb S positions (Figure 1A). In the electrophoresis at pH 6.2, this variant migrated discretely below the Hb S position (Figure 1B). The altered fraction of the alpha-chain (α^M) was observed in the polypeptide chain electrophoresis at pH 8.6 (Figure 1C).

Table 1. Hematological parameters for L.T.M. and reference values.

Hematological parameters	Values	Reference values
Erythrocyte ($\times 10^{12}/L$)	4.83	4.0-5.4
Hemoglobin (g/dL)	13.4	12-16
Hematocrit (%)	42.0	36-48
MCV (fL)	86.9	80-96
MCH (pg)	27.7	27-32
MCHC (%)	31.9	32-36
RDW-CV (%)	15.5	13.0-15.3

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW-CV = red cell distribution width-coefficient of variation.

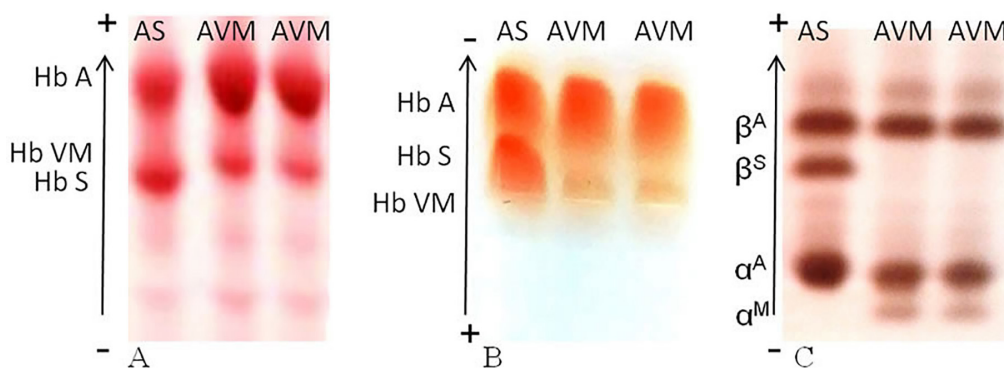


Figure 1. Electrophoretic mobility of hemoglobin (Hb) variant Val de Marne compared with Hb S. **A.** Electrophoresis at pH 8.6 on cellulose acetate tape. **B.** Electrophoresis at pH 6.2 on agar-agar gel. **C.** Polypeptide chain electrophoresis at pH 8.6 on cellulose acetate tape. Each picture depicts three samples: the first is a standard sample heterozygous for Hb S (Hb AS); the second and third are heterozygous for Hb Val de Marne (AVM). The arrows indicate the electrophoretic run direction.

The Hb VM chromatographic profile was similar in both HPLC systems presenting a retention time that was longer than for Hb A. Using the Ultra2 Resolution system (Trinity Biotech), the Hb VM was eluted at the relative retention time of S (0.98) with 13.4% concentration. We also observed a hybrid form of Hb VM, denominated Hb Val de Marne 2 (Hb VM2), at retention time S (1.07) with 0.6% concentration (Figure 2A). This pattern was compatible with the chromatogram of the Trinity Biotech variant library (Figure 2C). In the VARIANT™ system (Bio-Rad), the Hb VM appeared in the same window of Hb S elution with retention time of 4.41 and 14.7% concentration. The Hb VM2 appeared in an unknown window with retention time of 4.63 and 0.9% concentration (Figure 2B).

Regarding the concentration of the other Hbs, in the chromatogram using the Ultra2 Resolution system, the Hb A concentration was 66.8%, the Hb A₂ concentration was 1.8%, and the Hb F concentration was 0.0%. In the chromatogram performed using the VARIANT™ system, the Hb A concentration was 74.5%, the Hb A₂ concentration was 2.6%, and the Hb F concentration was 0.0%.

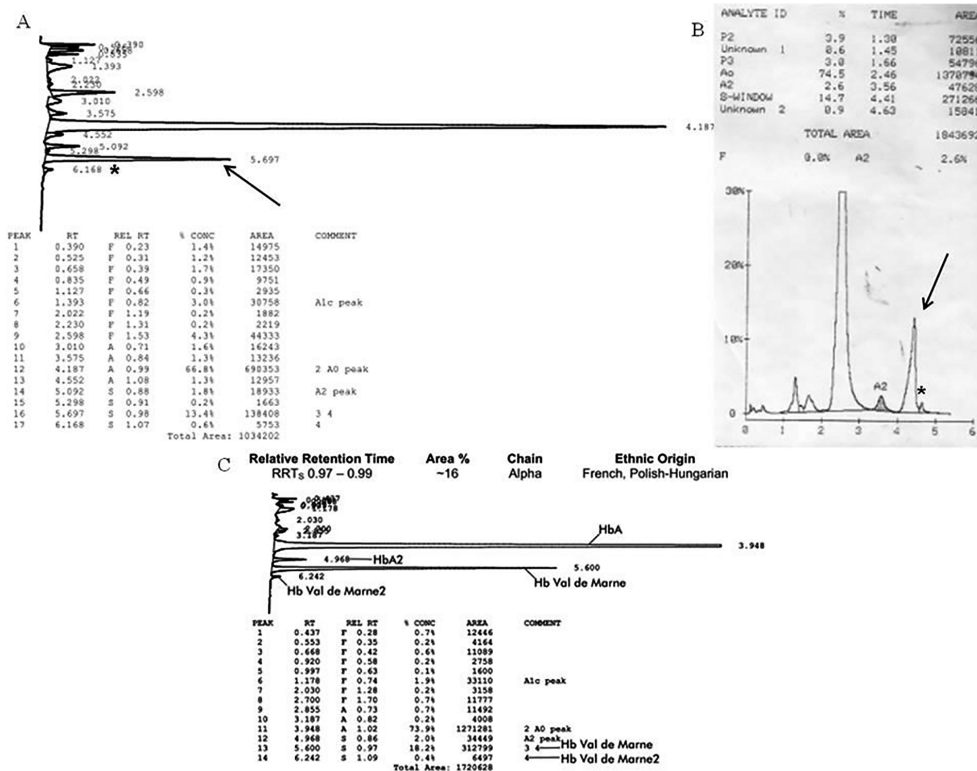


Figure 2. Chromatographic profile of hemoglobin (Hb) A/Val de Marne. **A.** Chromatogram performed using the Ultra2 Resolution system (Trinity Biotech). The Hb Val de Marne 1 peak appeared with 13.4% concentration at a relative retention time for S (RRT/S) of 0.98. The Hb Val de Marne 2 peak appeared with 0.6% concentration at an RRT/S of 1.07. **B.** Chromatogram performed using a VARIANT™ system (Bio-Rad). The Hb Val de Marne 1 peak appeared in the elution window of Hb S (4.41) with 14.7% concentration and the Hb Val de Marne 2 appeared in an unknown window with a retention time of 4.63 with 0.9% concentration. **C.** Chromatographic profile found in variant library made available by Trinity Biotech. The arrows indicate the Hb Val de Marne 1 peak and the asterisks indicate the Hb Val de Marne 2 peak.

DISCUSSION

The present study provides the first description of Hb Val de Marne in the American continent, more specifically in Brazil. The analysis of the hemoglobins and polypeptide chains in the electrophoretic profiles under different pH conditions, and the chromatographic profiles presented by the sample, allowed us to identify Hb Val de Marne and compare the results with other studies that have indicated this Hb variant in other populations (Wajcman et al., 1993; Owen and Hendy, 1994; Ma et al., 2004; Akbari and Hamid, 2012).

Wajcman et al. (1993) first described the Hb VM variant in a French family during a neonatal hemoglobinopathy screening program. The family resides in the Val de Marne Department situated to the southeast of Paris. In 1994, the same Hb variant was described by Owen and Hendy (1994) as a new Hb variant denominated Hb Footscray (owing to the region of Australia where it was discovered).

The description of the Footscray variant as a new Hb occurred because of the different patterns visualized in isoelectrofocusing (IEF), alkaline and acidic electrophoresis, and ion-exchange HPLC (Wajcman et al., 1993). Two newborns with alpha-chain Hb variants presenting IEF results with a slower migration pattern than Hb F were identified in the Department of Val de Marne. The parents of the children were examined and both the fathers, who were brothers, showed the same Hb pattern. One, a 39-year-old man, was investigated more closely. His red blood cell parameters were normal. Alkaline electrophoresis at pH 8.6 verified that the Hb VM migrated as two bands, one being more evident and formed by two mutated alpha-chains and two normal beta-chains (α_2^M/β_2^A), and the other less evident and formed by a hybrid (Hb VM2) of one normal alpha-chain with one mutated alpha-chain and two normal beta-chains ($\alpha^A\alpha^M/\beta_2^A$). In citrate-agar electrophoresis at pH 6.0, the Hb VM migrated below Hb A. The Hb VM concentration in this patient was 16.0%, the Hb A₂ concentration was 2.7%, and the Hb F concentration was 0.5%. The oxygen-binding properties verified that Hb VM presented a 1.7-fold increase in oxygen affinity (Wajcman et al., 1993). The same pattern described above was verified in a healthy 27-year-old male of Polish-Hungarian descent. The Hb VM concentration was 15.0% of the total Hb (Owen and Hendy, 1994).

Ma et al. (2004) presented the molecular characterization of a point mutation that comprised the substitution of a cytosine by an adenine in codon 34 of the *HBA1* or *HBA2* genes (AGC>AGA) causing the replacement of amino acid 133 in the protein (HBA:p.Ser133Arg). They found this mutation by automated DNA sequencing in a 15-year-old Chinese girl and her 61-year-old father, both without anemia. The pattern of Hb was determined by HPLC using a VARIANT II™ Hb Testing System (Bio-Rad Laboratories, Hercules, CA, USA) using the beta-thalassemia Short Program, and they found an Hb variant at the Hb S window with a concentration of 14.4%, Hb F with a concentration of <0.1%, and Hb A₂ with a concentration of 1.7% for the girl. The same Hb variant with a concentration of 16.0% was verified in the father's blood sample. The last report of the Hb VM variant was in a 26-year-old man living in Iran. Unlike in the other reports in which individuals had only one alpha gene altered, this man presented the heterozygous variant, or two abnormal *HBA* genes, with a percentage of Hb VM of 35.9% (Akbari and Hamid, 2012).

In the present case report, during alkaline electrophoresis, the Hb VM variant migrated between Hb A and Hb S, and it was not possible to detect the hybrid (Hb VM2), probably owing to its low concentration. During the agar-agar electrophoresis, the Hb VM variant migrated below Hb S. Both electrophoresis results suggest an S-like Hb variant. Therefore, we electrophoresed polypeptide chains at pH 8.6 and confirmed that the Hb variant was an alpha-chain mutant.

In the chromatogram obtained using the VARIANT™ system, Hb VM migrated as described by Ma et al. (2004) and it was possible to detect the presence of Hb VM2. In the chromatogram obtained using the Ultra2 Resolution system, the pattern was identical to the standard provided by Trinity Biotech in their Hb variant library. The electrophoretic and chromatographic results allowed us to confirm the presence of Hb VM and Hb VM2 subfractions in the sample under study.

Table 2 summarizes the observations described in the papers mentioned above according to the tests used to diagnose Hb VM.

Table 2. Parameters observed in studies that described the presence of Hb VM.

References	Subject	Local	IEF	Alkaline electrophoresis	Acidic electrophoresis	Polypeptide chain electrophoresis	Percentage of Hb variant
Wajzman et al. (1993)	39-year-old man	Val de Marne (France)	Slow moving below Hb A	Migrated as two bands below Hb A	Slow moving below Hb A	Abnormal alpha-globin band	16.0% ^{a*}
Owen and Hendy (1994)	27-year-old man	Footscray (Australia)	-	Migrated as two bands in the Hb F position	-	Abnormal alpha-globin band	15.0% ^l
Ma et al. (2004)	15-year-old girl ^h	China	-	Migrated as two bands in the Hb F position	Just one band: Hb A	Abnormal alpha-globin band	14.4% ^{l,ii*}
	61-year-old man ^a	China	-	Migrated as two bands in the Hb F position	Just one band: Hb A	Abnormal alpha-globin band	16.0% ^{l,ii*}
Akhari and Hamid (2012)	26-year-old man	Iran	-	-	-	-	35.9% ^l
Present study	32-year-old woman	Maringá (Brazil)	-	Migrated as one band below Hb A	Migrated as one band below Hb A	Abnormal alpha-globin band	14.7% (Hb VM) and 0.9% (Hb VM2) ^v 13.4% (Hb VM) and 0.6% (Hb VM2) ^v

^aDaughter and father; ^{*}retention time not reported; ^lquantification performed by ion-exchange high-performance liquid chromatography (HPLC); ⁱⁱpercentage determined by scanning the cleared cellulose acetate plate on a Beckman Model R-112 densitometer; ⁱⁱⁱquantification performed by HPLC on a VARIANT IITM Hb Testing System (Bio-Rad Laboratories, Hercules, CA, USA) using the Beta-Thalassemia Short Program; ^{iv}quantification performed by HPLC on a VARIANTTM Hb Testing System (Bio-Rad Laboratories) using the Beta-Thalassemia Short Program; ^vquantification performed by HPLC on an Ultra2 Resolution (Trinity Biotech, Wicklow, Ireland) with a Genesys assay kit.

For the identification of Hb variants, especially those with alteration alpha-chains, the use of several tests that are complementary to traditional Hb electrophoresis is necessary. This is important because several Hb variants called S-like variants exist with similar electrophoretic mobilities to Hb S. In the case of Hb VM, the chromatogram obtained using the VARIANTTM system suggested the possible presence of Hb S, but that was rejected because the concentration was below 15%, and because of the presence of an alpha-chain mutation in the polypeptide chain electrophoresis. The standard verification by chromatogram performed using the Ultra2 Resolution system left no doubt about the variant being studied.

Few studies have reported the presence of Hb VM, but its detection in different locations such as France (Europe), Iran (Asia), Australia (Oceania), China (Asia), and now in Brazil (South America) suggests a dispersion of this Hb variant. Because Hb VM brings no pathological consequences, patients do not seek medical care and thus remain undiagnosed.

Certainly, L.T.M.'s European ancestry contributed to her inheritance of Hb VM, taking into account the presence of Hb VM in Italy and Ukraine. Both Italians and Ukrainians contributed to the ethnic composition of Brazil because both came to Brazil from 1870 onwards in search of employment in plantations, mainly in the South, to replace slave labor (Zamberlam, 2004).

The discovery of the Hb VM variant in 1993 is recent. Therefore, little is known about the characteristics of Hb with regards to its hematological profile and the pattern it adopts in diagnostic tests for Hb variants. Thus, this study is important because it reports for the first time the Hb VM variant in the Americas, and presents the results of electrophoretic and chromatographic procedures to facilitate the diagnosis of the Hb variant described.

Conflicts of interest

The authors declare no conflict of interest.

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