Compound heterozygosity of Hb Q\textsuperscript{India} (α\textsuperscript{64} (E13) ASP⇒HIS) and -α\textsuperscript{3,7} thalassemia. First report from Argentina

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ABSTRACT

Hemoglobin (Hb) Q-India is an innocuous α-globin variant: α\textsuperscript{64} Asp⇒His. DNA sequencing studies have shown that the Hb Q India mutation is GAC⇒CAC in codon 64 of the α\textsubscript{1} gene. Hb Q-India is a well-known hemoglobin variant in South-East Asia but only isolated case reports exist in literature to describe this rare entity in the rest of the world. The variant has been found with various forms of α and β thalassemia. This hemoglobin has the same electrophoretic mobility as Hb S. We report, for the first time, the identification of Hb Q-India in an Argentinian woman (her parents came from Gibraltar), referred to our laboratory bearing a mild microcytic hypochromic anemia; a co-inherited α\textsuperscript{+} thalassemia (-α\textsuperscript{3,7} th) was also found.

Key words: Abnormal hemoglobin (Hb), microcytic hypochromic anemia, Hb Q India.

RESUMEN

La hemoglobina (Hb) Q India es una hemoglobina anormal e inocua que afecta la cadena α de esta. Los análisis de secuencia han demostrado que la mutación se encuentra en el codon 64 GAC⇒CAC del gen α\textsubscript{1}. Si bien es una variante muy conocida en el sudeste asiático, solo se han reportado pocos casos en el resto del mundo. Esta hemoglobina anormal se ha encontrado asociada con diversas formas de α y β talasemia y su posición electroforética es idéntica a la de la Hb S. Reportamos, por primera vez, la identificación de
Hemoglobin (Hb) Q disorders are an important group of hemoglobinopathies. Several variants including Hb Q Thailand, Hb Q Iran and Hb Q India are documented. Only isolated case reports exist in literature to describe this rare entity. They occur normally in the heterozygous form and could be associated with thalassemia. The three Hb Q variants do not cause hematological disorders because the residues involved are on the surface of the hemoglobin tetramer and charge changes at these positions do not affect the properties of the hemoglobin molecule. This particular Hb Q variant, Hb Q-India, is an \( \alpha \) chain variant: \( \alpha_{64} \) Asp \( \rightarrow \) His. DNA sequencing studies have shown that the Hb Q India mutation is GAC \( \rightarrow \) CAC in codon 64 of the \( \alpha_1 \) gene. We hereby report, for the first time, the identification of Hb QA India in an Argentinian women, in association with \( \alpha^+ \) thalassemia (-\( \alpha^{3,7} \) deletion), referred to our laboratory bearing a mild microcytic hypochromic anemia.

Hematological data were obtained with a Coulter Counter model ACT10 (Coulter Corporation, USA). Hb A\(_2\) was measured by elution post electrophoresis at alkaline pH, and Hb F according to the method described by Betke et al. Isopropanol (Carrell & Kay) and heat stability tests were performed. Cellulose acetate electrophoresis at alkaline pH; citrate agar electrophoresis at pH 6; and electrophoresis at alkaline pH of globin chains, were carried out using standard methods. Sickling test was negative. The isopropanol an the heat test were also negative indicating the absence of an unstable Hb.

Cellulose acetate (alkaline pH) electrophoresis detected Hb X moving to Hb S position and citrate agar (acid pH), moving Hb X as a sharp band close to Hb A. Globin chain electrophoresis enables the identification of an alpha chain alteration.

The hematological data of the patient are shown in [table].
DNA was extracted from peripheral blood cells by standard methods.\textsuperscript{12}

The sample was analyzed by allele specific amplification of -α\textsuperscript{3.7} deletion.\textsuperscript{13} Selective amplification of the α\textsubscript{2}-globin gene was performed in a thermal mini cycler (MJ Research, Watertown, MA). Amplification was accomplished according to conditions already described.\textsuperscript{13}

Polymerase chain reaction (PCR) was carried out and then a direct DNA sequencing of the PCR products was performed. PCR amplification of the α\textsubscript{2}- and α\textsubscript{1}-globin genes was accomplished by using oligonucleotide primers (CyberSyn, Lenni, PA, USA); the common forward primers FAa2:

\[5'\text{ACGCGCTCGCGGCCCGGACACA}3',\]

and reverse specific primers for the α\textsubscript{2} gene:

\[5'\text{AGGGAGGCCCATCGGGCAGGAGGAA}3',\]

and α\textsubscript{1} gene: \[5'\text{AGGGGGGAGGCCCAAGGGGCAAGAAA}3'.\]

Reverse primers and reaction conditions used are the ones described to detect α-thal-2 (-3.7 kb).\textsuperscript{14} Sequencing was done using a Big Dye Terminators Ready Reaction Kit (Perkin-Elmer Cetus, Norwalk, CT, USA) in an ABI PRISM 310 sequencer (Perkin-Elmer Cetus). Primers used for sequencing the two genes were the following: exon 1, common forward primers FAa2; exon 2, primer S2

\[5'\text{CCCACCACCGCCAGCCACCA}3';\] exon 3, primer S3

\[5'\text{GCACGACGGGCCAGGAGGAGG}3'.\] The reverse specific primers for the α\textsubscript{1} gene were used to confirm the mutation (fig.).

\begin{table}
\centering
\begin{tabular}{|l|l|}
\hline
Parameters & Prbband \\
\hline
Hb A/K/\(\alpha_2\) (\% Alkaline pH) & 20 \\
Hb (g/L) & 119 \\
RBC (10\textsuperscript{12}/L) & 5,23 \\
MCV (fl) & 71 \\
HCM (pg) & 22 \\
Reticulocytes (\%) & 3,8 \\
Hb A\textsubscript{2} (\%) & 2,2 \\
Hb F (\%) & 1,8 \\
Isopropanol Test & negative \\
Heat Test & negative \\
Sickling Test & negative \\
\hline
\end{tabular}
\caption{Hematological data}
\end{table}
Molecular biology studies (PCR) showed the presence of $-\alpha^{3,7}$ deletion, and the sequencing of $\alpha$-1 gene showed a $\text{GAC} \rightarrow \text{CAC (Asp} \rightarrow \text{His)}$ substitution at codon 64, corresponding to Hb Q India (fig.). Even though Hb Q India is detectable by electrophoresis its correct characterization requires high-performance liquid chromatography (HPLC) or molecular analysis.

It is the first time that this abnormal hemoglobin is described in our country. The low red blood cell indexes observed in this case could be due to co-inheritance of $\alpha^+\text{ thalassaemia} (-\alpha^{3,7}\text{ deletion}).$

**REFERENCES**


Recibido: 24 de marzo del 2010.
Aprobado: 12 de abril del 2010.

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