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Abnormal hemoglobin11 - Hb (Karachi), An a-Chain Abnormality at Position 5 Ala Pro

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ABNORMAL HEMOGLOBINS[11] - Hb (KARACHI), AN a CHAIN ABNORMALITY AT POSITION 5 ALA PRO

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Abstract

Hemoglobin Karachi is a new a chain Variant with amino acid substitution a 5 (A3) Ala-“ Pro. The hematological data on the propositus were normal. In cellulose acetateelectro phoresis this hemoglobin migrated towards the anode slower than HbA.
It is the first a-chain abnormal hemoglobin reported from Pakistani population (JPMA 36:206, 1986).

INTRODUCTION

Numerous structural abnormalities of the globin chains have been reported since Pauling et al.[1] discovered the first abnormal hemoglobin, sickle cell hemoglobin. Amino acid sequencing later revealed it to contain a substitution of valine for glutamic acid in the 6th position of the B chain.[2] Extensive researches for abnormal hemoglobin’s in many parts of the world have brought to light over 450 structurally altered hemoglobins. They include HbJ Toronto characterized by replacement of an alanine by aspartic acid in position 5 of the a chain[3]; Hb Sawara[4] Hb Ferndown,[5] Hb Dunn,[6] Hb Woodville,[7] and Hb Swan river[8] in which aspartic acid in position of a chain is replaced by Ala, Val, Asn, Tyr and Gly respectively. These abnormalities are all present in the helical region A of the a chain. An abnormal hemoglobin of the same helical region was discovered incidentally in a 24 year old healthy male who was used as a control during a study on abnormal hemoglobins. In his case, alanine is replaced by proline in position 5 i.e. helical region A 3. This new variant has been designated Hb (Karachi).

MATERIALS AND METHODS

Blood samples from the propositus, his mother and two sisters were collected in EDTA and standard hematological studies were carried out. Hemolysates were prepared by the toluene method[9] and globin was separated according to. Anson and Mirsky’s procedure.[10] The electrophoresis of the hemoglobin was carried out on cellulose acetate membranes (Cela gram II, 78 x 150 mm), in Tris-EDTA- borate buffer pH 8.4for 30 min at 350 volts[11] followed by staining with Amido black lOB[12] and Ponceau.[13] The globin chains were separated on a CM-Cellulose column (13 x 1 cm) with buffer containing 8M Urea, 0.05M 2-mercaptoethanol and Na2 HPO4 pH 6.5 to 7.2.[14] The peaks separated were checked for homogeneity by electrophoresis on polyacrylamide gels in Triton x-100.[15]
The separated a chain was digested with trypsin (Worthington Tos Phe CH2CI treated) at room temperature, pH 8.5; after 3 hrs the reaction was stopped by addition of 0.5 M HCI.
The tryptic peptides were separated by high voltage electrophoresis (Pherograph, Hamburg, West Germany) in 0.02M pyridine acetate buffer pH 4.7 for 2 hrs at 2000 volts and chromatography (Butanol: acetic acid: water: pyridine, 15:3:12:10) to prepare two dimensional peptide maps (fingerprint.[16,17])The peptides were visualized by stronsium ninhydrin spray.[18]

Amino acid analysis of a chain was carried out on an automatic amino acid analyser (Biotronik GmbH Munich, West Germany).

The amino acid sequence of the a chain were determined by automatic Edman degradation[19] in a Beckman sequencer 890C (Palo Alto California) by using Quadrol programme (Quadrol concentration 0.25M) and manual sequencing by DABITC method. [20,21]

RESULTS

Hematological examination on the propositus revealed the following: Hemoglobin 15.2g/dl; red cell count 5.lxl0^{12} /1; reticulocytes 0.5%; PCV 47%; MCV 92fl; MCH 30 pg; MCHC 32%; total biirubin 13 u mol/1; serum iron 106 mcg/dl; TIBC 380 mcg/dl. Erythrocyte morphology was normal. Incubation of fresh red cells with brilliant cresyl blue disclosed no inclusion bodies within the cells. The osmotic fragility test revealed 50% hemolysis at 0.43% NaC1, being within normal range. A heat stability test[22] gave normal results.

The electrophoretic pattern of Hb (Karachi) on cellulose acetate membrane at pH 8.4 is shown in Figure 1.

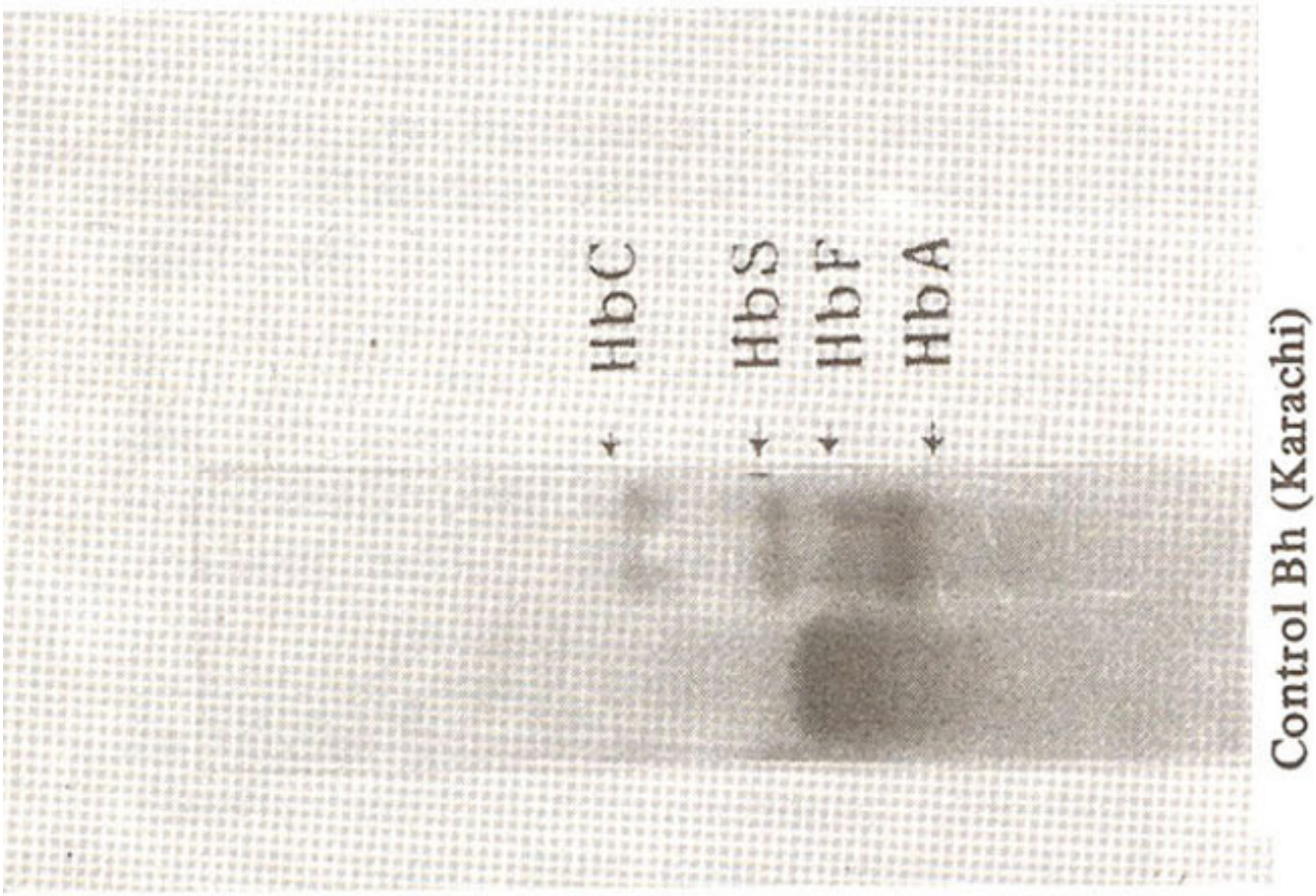

Figure 1. Electrophoretic pattern of hemoglobin on Cellulose acetate membrane. Tris-EDTA-borate buffer, pH 8.4.

It is slower than HbA. Neither the mother nor the two sisters of the propositus showed any abnonnal

bands in electrophoresis of their hemoglobin.
Chromatography of Hb (Karachi) on CM-Cellulose revealed 3 peaks (Fig.2).

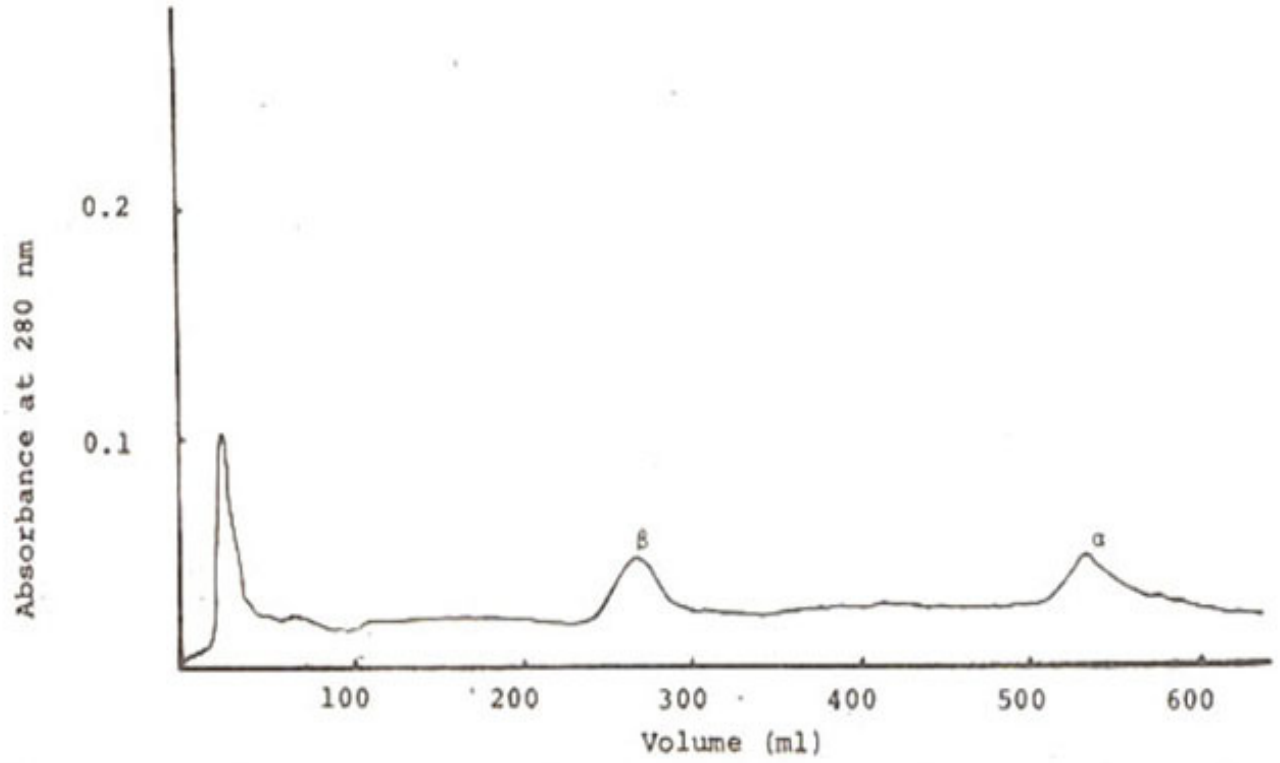

Figure 2. Separation of the polypeptide chains from Hb (Karachi) on CM-Cellulose column. Elution buffer: 8M Urea + 0.05M 2-Mercaptoethanol + 0.01M Na_2HPO_4; pH 6.5-7.2.

The first peak contained unbound material followed by the B and a chain. There was no other peak detected. The amino and composition of c chain was found to be normal whereas slight elevation of praline was nosed in a chain.
The fingerprinting of a chain showed a change in its position of peptide 1 (Fig 3).

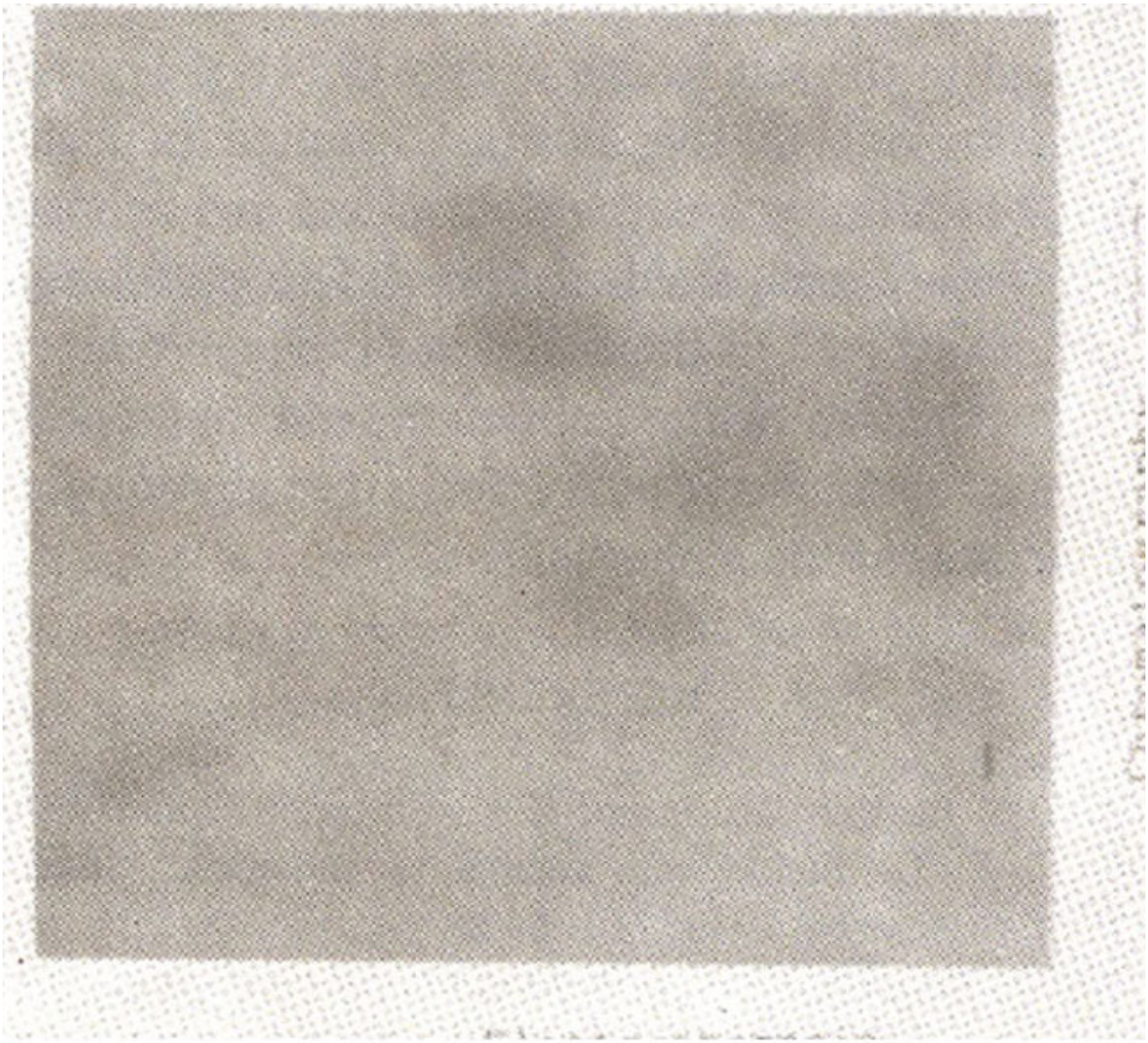

Figure 3. Fingerprint of the α chain from Hb (Karachi) digested with TPCK-trypsin on Wattmann paper 3.
Electrophoresis in 0.02M pyridine acetate, pH 4.7. Chromatography in Butanol: acetic acid: water:pyridine (15:3:12:10).

The amino acid sequence of the whole a chain (upto 30 steps) on sequencer and on manual sequencing (10 steps) showed a replacement at position 5 of Ala by Pro. There was no trace of Ala in this position suggesting that the hemoglobin has a_2 K B_2 and the individual carrying a homozygous variant.

Hb (Karachi) a chain Val Leu Ser Pro Pro Asp
LYs Thr Asn Val
Hb A a chain val Leu Ser Pro ALa Asp

LYs Thr Asn Val

DISCUSSION

The hematological data showed all values to be in the normal range. The structural studies of the a chain show that alanine residue at 5th position of the a chain is substituted by a proline residue. Alanine is ambivalent i.e. neither so hydrophobic that it always point internally in the hemoglobin molecule nor so hydrophiic as to point externally. Alanine in the 5th position of the a chain, corresponding to the 3rd residue of the A helix is oriented towards the surface of molecule. Its substitution by proline which has the same ambivalent characteristics may not produce any change in structure-function relationship. The a carbon of the side chain of proline loops back to make a second connection to its amine nitrogen, producing a bend in the main polypeptide chain. This is in apposition to the A 2 proline residue in the normal hemoglobin. The only other mutation in the helix A 3 position reported is in HbJ Toronto, in which alarline is replaced by strongly hydrophilic aspartic acid. At helix A4 position as many as 5 mutations are known. This position is involved in the salt bridge with a 127 (HlO) lys and substitutions in this position resulted in higher oxygen affinity but the Bohr effect and cooperativity were comparable to HbA.[5] The position A3 is not involved in the function of the molecule; as such changes with ambivalent and hydrophiic amino acids may not affect the physiological functions of the molecule.

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