A Novel Double Heterozygous Hb D-Punjab/Hb J-Meerut Hemoglobinopathy

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Abstract A comprehensive laboratory diagnosis of hemoglobinopathies forms an integral part in workup of disorders of globin chain synthesis. Clinical findings, complete blood counts, peripheral smear examination along with hemoglobin (Hb) electrophoresis and/or cation exchange high performance liquid chromatography findings and parental study helps to clinch a final diagnosis. Compound heterozygous hemoglobinopathy presents with variable clinical findings and some of them are picked up on screening tests done as part of routine antenatal workup. Here we report a rare double heterozygous hemoglobinopathy of Hb D-Punjab and Hb J-Meerut in a 35 year antenatal female.

Keywords Hemoglobinopathy · Double heterozygous Hb D-Punjab · Hb J-Meerut

Case Report

A 35 years old pregnant female, resident of Uttar Pradesh came for antenatal screening. She was 4th gravida with 12 weeks of gestation with previous two abortions and one live issue. There was no past history of jaundice or blood transfusion and no significant family history was found. Her hematological parameters, recorded by automated,

Sysmex XT1800i, were as follows: hemoglobin (Hb) 11.0 g/dl, red blood cell count (RBC) 4.53 million/cumm, hematocrit (HCT) 35.0%, mean cell volume (MCV) 77.3 fl, mean cell hemoglobin (MCH) 24.3 pg, mean cell hemoglobin concentration (MCHC) 31.4 g/dl, red cell distribution width (RDW-CV) 15.4%. Peripheral smear showed predominantly normocytic normochromic red cells with few microcytic hypochromic red cells. Reticulocyte count was 1.5%.

Hemoglobin capillary zone electrophoresis (Hb-CZE) was carried out using automated Capillary Mini Flex Piercing analyzer, Sebia, France using alkaline buffer pH 9.4. Hb-CZE indicated the presence of four peaks as follows: Hb A (52.2%), Hb D (23.3%), Hb A2 (1.9%) and unknown peak of 22.6% in zone 12 (Fig. 1). The zone 12 in Hb-CZE depicts early peaks that can be seen in Hb J-Meerut. Identification and confirmation of variant peak was done in conjunction with cation-exchange high performance liquid chromatography (CE-HPLC) results. On further analysis on HPLC (Bio-Rad Variant II β thalassemia short programme analyzer, Bio-Rad Laboratories, CA, USA), HPLC showed peak within the P3 retention time window (20.4%) with HbA—50.0%, HbD—22.0%, HbA2—1.6%, HbF—0.2%, P1—0.4%, P2—1.9% and others—3.5% (Fig. 2). The common hemoglobins that elute in P3 window are fast moving hemoglobins (FMH) that are α and/or β chain variants. However, in India Hb J-Meerut is found to elute at RT $\sim$ 1.77 min. Hb H usually elutes at <1 min on HPLC and migrates at zone 15 in Hb-CZE. Also, since no golf ball inclusions were seen on reticulocyte preparation, we could exclude the possibility of Hb H disease. Hence, in this case a diagnosis of double heterozygous for Hb J-Meerut and Hb D-Punjab was rendered. Spousal HbCZE revealed normal Hb-electrophoretogram.

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Fig. 1 Capillary zone electrophoresis of hemoglobin shows presence of four peaks: Hb A (52.2%), Hb D (23.3%), Hb A2 (1.9%) and unknown peak of 22.6% with migration to zone 12

Fig. 2 Hemoglobin CE-HPLC showed peak within the P3 retention time window (20.4%) with Hb D—22.0%, HbA—50.0%, HbA2—1.6%, HbF—0.2%, P1—0.4%, P2—1.9% and others—3.5%

Discussion

Hb D-Punjab is a β chain variant caused by a point mutation (β 121 Glu $\rightarrow$ Gln) [1]. Hb J-Meerut is a fast-moving hemoglobin (FMH) on electrophoresis [2]. It is an α -globin chain variant (α 120 Ala-Glu) that elutes in P3 window on CE-HPLC and migrates to zone 12 on Hb-CZE [3]. Both heterozygous HbD and heterozygous Hb-J are clinically silent conditions. Co-occurrence of HbD Punjab with Hb J-Meerut is unusual.

The case is presented for its rarity and unique features. The clinical presentation in this case is bad obstetric history

that cannot be explained by double heterozygous hemoglobinopathy alone. Local and endocrinal causes need to be further ruled out. Also, co-existence of both α and β chain variants can lead to uncharacterized peaks composed of variant α and β chains on HPLC/electrophoretograms [4]. However occurrence of such peaks is unpredictable and in this case it may have migrated with HbA on Hb-CZE.

This case also highlights the need to harmonize two easily available i.e., CE-HPLC and Hb-CZE techniques to help in final diagnosis where molecular workup is not sought after primarily.

Compliance with Ethical Standards

Conflict of interest None.

Research Involving Human and/or Animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was taken from the subject.

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