

## EDUCATIONAL ARTICLE

### HEMOGLOBIN VARIANTS DETECTION BY HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) METHOD

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The hemoglobin molecule is composed of a prosthetic heme group attached to two pairs of unlike polypeptide chains. Normal adult hemoglobin (A) consists of two  $\alpha$  and two  $\beta$  chains ( $\alpha_2\beta_2$ ). A second normal adult hemoglobin (A2) consists of two  $\alpha$  and two  $\delta$  chains ( $\alpha_2\delta_2$ ). The primary structure of the  $\delta$  chain differs from that of the  $\beta$  chain at ten amino acid positions. The blood of normal adult humans contains both A as the major hemoglobin species and A2 as a minor hemoglobin species. Human fetuses and newborn infants produce mainly fetal hemoglobin (HbF) which consists of two  $\alpha$  chains and two  $\gamma$  chains. The  $\beta$  and  $\gamma$  chains differ at 39 amino acid positions. Additionally, the  $\delta$  chain and the  $\epsilon$  chain have been observed in early human embryos. Newborn infants have two types of G chain, the G  $\gamma$  chain which has a glycine residue at position 136 (HbG2), and the A $\gamma$  chain which has an alanine residue at position 136 (HbG1). Thus, seven types of hemoglobin chains exist in the human, namely  $\alpha$ ,  $\beta$ ,  $\gamma$ , A $\gamma$ , G $\gamma$ ,  $\delta$ , and  $\epsilon$  chains.

More than 900 hemoglobin (Hb) variants are currently known. Worldwide, an estimated 150 million people carry Hb variants (1) and hemoglobinopathies are the commonest inherited disorders, constituting a significant healthcare problem. (2) Therefore, reliable detection and identification methods are essential. Common techniques used in Hb analysis are electrophoretic and chromatographic assays. Electrophoresis of hemoglobin variants with similar mobilities has inherent limitations. The identification of variants is dependent on the technical performance of electrophoresis, which has many variables, e.g., hemoglobin concentration, amperage, running temperature, and length of electrophoresis run. These variables can affect the quality of separation and relative positioning of the bands. Variants that migrate identically or similarly would be very difficult, if not impossible, to evaluate without the unknown sample being electrophoresed directly adjacent to the reference hemoglobin mixture or adjacent to several known stored specimen. Alkaline and acid hemoglobin electrophoresis are the two most widely used methods for investigating hemoglobin variants and hemoglobinopathy. Alkaline electrophoresis is rapid, reproducible, and capable of separating common hemoglobin variants, such as hemoglobin A (HbA), HbF, HbS, and HbC, but HbS, HbD, HbG, and HbLepore are unresolved from each other, as are HbC, HbA2, HbO-Arab, and HbE. In addition, there are other variants with electrophoretic mobilities identical or similar to those of HbS and HbC. Consequently, acid electrophoresis is needed for the identification of the aforementioned variants. Nevertheless, these electrophoretic methods are still not able, in most cases, to separate HbD from HbG and HbLepore and, in some cases, HbE from HbO-Arab. (3) Hemoglobin fraction analysis by cation-exchange HPLC has the advantage of quantifying HbF and HbA2 along with hemoglobin variant screening

in a single, highly reproducible system, making it an excellent technology to screen for hemoglobin variants and hemoglobinopathies along with the thalassemias. (4) The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of hemoglobin fractions makes this an ideal methodology for the routine clinical laboratory. (5) Numerous automated HPLC systems are now commercially available, and evaluations have been published. (6)

HPLC has been shown to have a high degree of reproducibility and precision. HPLC has made hemoglobin abnormality detection much more accurate, faster, and automated. Screening by HPLC has enabled identification of couples at risk of giving birth to thalassemia major child. In addition, HPLC identifies individuals with clinically-silent hemoglobin variants like Q India, J, D Iran, and C. Recent advances in manufacturing technology have helped in better detection of hemoglobin variants by incorporating minute differences in the retention time. The use of HPLC technology in the clinical laboratory setting has increased 12.5-fold in the past 10 years. (7)

#### HOW HPLC TECHNIQUE WORKS

The process of HPLC starts with extraction of analyte of interest for liquid chromatographic process. The extract is forced through a column usually a small tube packed with small round particles with a certain surface chemistry (stationary phase) by pumping a liquid (mobile phase) at high pressure of 20-200 kg/cm<sup>2</sup> through the column. The stationary phase is usually in the form of small-diameter (5-10  $\mu$ m) uniform particles, packed into a cylindrical column. The extract is introduced in a small volume to the stream of mobile phase. The amount of retardation depends on the nature of the analyte, stationary phase, and mobile phase composition. The typical column is constructed from a rigid material (such as stainless steel) and is generally 5-30 cm long with an internal diameter in the range of 1-9 mm. HPLC is preferred due to its high speed, better resolution, sensitivity, reproducibility, accuracy (99 percent), and automation. With today's advanced instrumentation and chemistry, the technique has earned its justified name high performance liquid chromatography.

#### CONCLUSION

In conclusion, HPLC is an excellent, powerful diagnostic tool for the direct identification of hemoglobin variants with a high degree of precision in the quantification of major and minor, normal and abnormal, hemoglobin fractions. HPLC is suitable for the routine investigation of hemoglobin variants, hemoglobinopathies, and thalassemia. With the integration of proper algorithms (involving retention time, %Hb, and peak characteristics) a clinical laboratory is capable of identifying 75% of the common

variants encountered without the need for confirmatory studies such as alkaline and acid electrophoresis. More importantly, identification of the common variants (i.e., HbC, HbD-Punjab, HbE, HbG-Philadelphia, HbHope, HbLepore, HbO-Arab, and HbS) that in combination with HbS leading to a clinically significant sickling disorder can be quickly and accurately accomplished by use of such algorithms without the need for further testing.

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## EDUCATIONAL ARTICLE

### THYROID MALIGNANCIES IN CHILDREN

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#### Abstract

Thyroid malignancies in children are a known and distinct entity, though is less common than the thyroid malignancies in adults. The children tend to be treated in a manner similar to the adults with thyroid cancers, although there are striking differences in the presentation, clinical behavior, the differentiation pattern of the tumor and the outcome of management. Since the number of affected children are scarce and dispersed over wide regions, it is difficult to study these tumors in great detail. This article provides a review and comparative analysis between the adult and pediatric thyroid malignancies, thus guiding us in formulating appropriate approach for children.

#### Introduction

The Chernobyl tragedy in April 1986 in USSR showed the world the gruesome picture of occurrence of thyroid cancers in children which was otherwise seen very rarely (1) . According to the Chernobyl Forum, many years after the incident, about 4000 new cases of thyroid cancers occurred because the children consumed the cow's milk and the leafy vegetables contaminated with radioactive iodine, apart from the radiation effects of radioactive material on the thyroid (2).

In our clinical practice, albeit rarely, one does encounter nodules of the thyroid gland in children

which could be malignant. Since not much of research in terms of prospective randomized trials has been undertaken for the malignancies of thyroid gland in children, it is still a subject less well understood by clinicians.

#### Comparative Analysis

The salient features of malignancies of thyroid in children are that all children, especially those who are <10 years of age at presentation with thyroid nodules must be investigated thoroughly and a histopathological diagnosis established rapidly so that appropriate treatment is instituted at the earliest. An attempt is made here to provide a comparison between the adult and the pediatric thyroid malignancies with a view of improved understanding of the lesion (Table 1).

There are not many diagnostic modalities to differentiate between benign and malignant thyroid nodule (as depicted in Table 2). A baseline ultrasonography and a rapid histopathological evaluation by a Fine Needle Aspiration Biopsy (FNAB) or rarely open surgical biopsy (depending on the size of the lesion) is most essential.

The most widely accepted treatment modality is surgical excision of the lesion with excision of grossly involved lymphatic system, though a radical neck dissection is not recommended by all authors.