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Paper No. : 08 Human Population Genetics
Module : 22 Haemoglobinopathies

ज्ञान-विज्ञान विमुक्तये

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Description of Module	
Subject Name	Anthropology
Paper Name	08 Human Population Genetics
Module Name/Title	Haemoglobinopathies
Module Id	22

Learning outcomes

- You shall be able to learn the history of Haemoglobin research.
- You shall be able to understand the Haemoglobin molecule and the globin genes that play an important role in our body.
- You would know the meaning of Haemoglobinopathies, how to identify it and what are the endemic and the sporadic haemoglobin variants.

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1. Introduction

Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs. The normal function and total amount of Hb depend on its adequate synthesis and precise structure. When these conditions are not met, the red cells of the carrier contain either a decreased amount of Hb or an Hb variant that may have abnormal properties. These conditions are collectively described as haemoglobinopathies.

2. History of Haemoglobin Research

Human haemoglobin started with the investigations on sickle cell anemia; a hereditary disease. Herrick observed a sickle-shaped abnormality of red cell structure in an anemic black student in 1910. Taliaferro and Huck (1923) recognized that the condition is hereditary. Neel (1949) and Beet (1949) had shown that patients with sickle cell anemia are homozygous for a gene that is in the heterozygous state, causes much milder condition: sickle cell trait which was found in about 8% of the American black population.

The decisive step in the biochemical-genetic analysis of this disease was carried out by Pauling et al (1949) in a paper with the programmatic title, "Sickle Cell Anemia, a molecular disease." The authors examined the haemoglobin of patients with sickle cell trait and sickle cell anemia, comparing them with the haemoglobin of normal individuals. In accordance with the state of methodology for protein analysis at that time, these investigations were performed using the Tiselius zone electrophoresis. The results conclude with a significant difference exists between the electrophoresis mobility of hemoglobin derived from erythrocytes of normal individuals and from those of sickle cell anemic individuals (Pauling et al 1949).

The gene in Sickle cell anemia is in homozygous state whereas the carriers of the sickle cell trait are heterozygous. This reveals that a change produced in a protein molecule by an allelic change in a single gene involved in synthesis. In 1956, Ingram working in Cambridge in the laboratory where Perutz was pursuing his crystallographic work, Sanger had shown the amino acid sequence of insulin, and Crick and Watson had demonstrated the DNA model. The fingerprinting method of protein analysis had revealed that sickle cell hemoglobin was identical with the normal molecules in all peptides except one differing peptide where glutamic acid was replaced by valine.

Meanwhile, after new methods of electrophoresis had replaced the cumbersome Tiselius electrophoresis, many other hemoglobin variants were also discovered. At present, over 400 of such variants are known. Further steps of great importance were the establishment and elucidation of the full amino acid sequence of the hemoglobin chains by Braunitzer et al. 1961, and of the three dimensional structure of hemoglobin. The isolation of the haemoglobin mRNA led to new insights into gene structure and function and opened up new paths to the understanding of gene action.

Molecular work on the haemoglobin has proceeded at a rapid rate. The full DNA sequences of the various haemoglobin genes and their flanking sequences is now known, and the haemoglobin genes are better understood than any other mammalian genes. Mutations affecting the haemoglobin, particularly the thalassemias, have been elucidated and are models for the understanding of gene action at the molecular level.

3. Haemoglobin molecule

3.1. Types of normal Haemoglobin in humans

There are four types of haemoglobin which appear in human lifetime. These are as follows:

1. The embryonic haemoglobin (Hbs Gower I, II and Hb Portland), which are detectable from the third to the tenth week of gestation.
2. The fetal haemoglobin (HbF), the major haemoglobin at the time of birth.
3. The adult haemoglobin (HbA).
4. The minor adult haemoglobin (HbA_2).

During the first year of life, HbF is replaced by HbA and HbA_2 where a normal adult man contains 98% of HbA and 2% of HbA_2.

3.2. Haemoglobin structure

The haemoglobin molecule consists of two pairs of identical amino acid chains that interweave in space, forming an ellipsoidal tetrahedron; its molecular weight is approximately 64000Da. There are several types of chain; each has a precise amino acid sequence that is fully conserved from generation to generation. The various chains display a marked sequence similarity: they have similar amino acids in several positions across their sequence, implying a common ancestral form. The type of hemoglobin molecule is determined by its constituent chains. Adult hemoglobin (HbA_2) is composed of two α peptide chains, each consisting of 141 amino acids and two β peptide chains, each consisting of 146 amino acids whereas in minor adult haemoglobin two β peptide chains is replaced by δ chains. The fetal haemoglobin has two γ peptide chains instead of β peptide chains. (Figure 1 summarizes the basic information on various hemoglobin species in relation to the location of their genes on the chromosome).

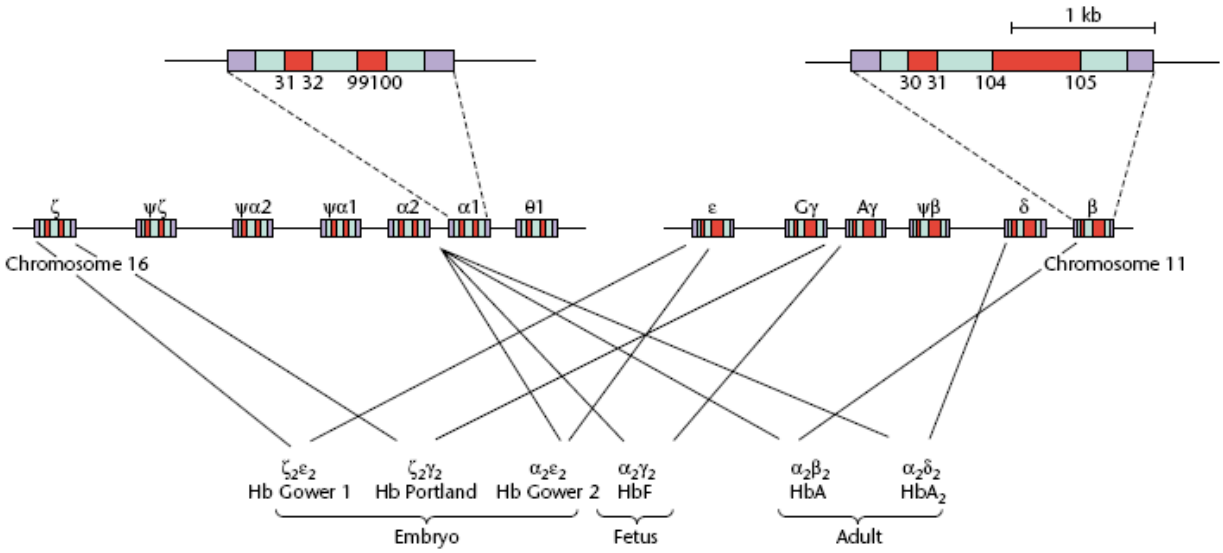

Figure 1: Chromosomes 16 and 11, the globin gene clusters and the respective chains. The genes are arranged (from 5' to 3') according to the timing of their activation. Green sections represent translated sequences (exons) and red sections denote intervening sequences (introns). Blue sections correspond to the untranslated 5' to 3' sequences flaking the globin genes. Combinations of the chain produced result in the various types of haemoglobins described in the text. Retrieve from Weatherall, 1991.

3.3. Haemoglobin function

The structure of the haemoglobin is complicated which makes the molecule to have its accurate and long lasting function for example each haem acquires an oxygen molecule which is inserted between the respective distal histidine and iron when the red cells pass through the lung capillaries (where the oxygen tension (Po_2) is 60mmHg). The four haems bind and release oxygen sequentially. Moreover, binding of oxygen by one haem 'facilitates' the binding (or release) of oxygen by the next haem; this allosteric function is shown graphically (Figure 2) by the sigmoidal shape (Dimitris L Loukopoulos, 2002) of the oxygen equilibrium curve, which displays: (1) a horizontal part when the Po_2 varies around 60–80mmHg, reflecting the adequate saturation (close to 100%) at all potential fluctuations of the oxygen pressure in the air, the airways and the alveoli); (2) a steep oblique part for Po_2 values, decreasing from 60 to 20 mmHg, thus ensuring a satisfactory gradual release of oxygen when red cells pass through the tissues; and (3) a final horizontal part at partial oxygen pressures lower than 20mmHg.

Equally important is the fact that, on binding oxygen, each chain undergoes a redistribution of the low-energy bonds and slightly changes its shape; as a result, the whole Hb tetramer acquires an 'R' (relax) conformation on oxygenation, and squeezes into a 'T' conformation on giving up oxygen (Dimitris L Loukopoulos, 2002). The latter change and release of oxygen is facilitated by the introduction of a 2,3-

diphosphoglycerate molecule (2,3-DPG) in the central cavity. 2,3-DPG is a product of the Rapaport–Luebering shunt of the glycolytic Embden–Meyerhof pathway, and its concentration is adjusted, to a certain degree, depending on tissue needs for oxygen (Dimitris L Loukopoulos, 2002).

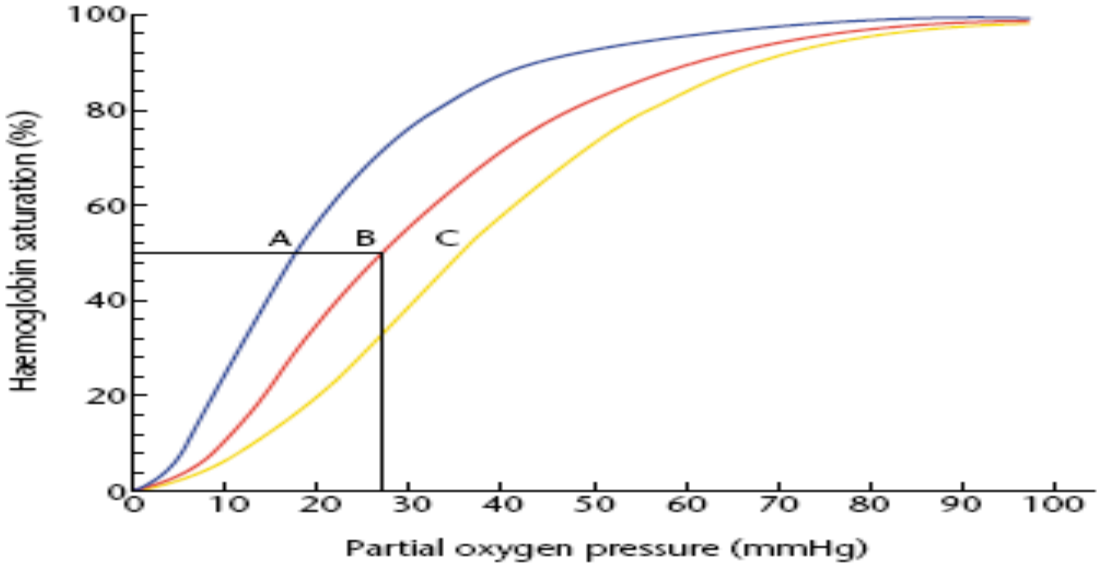

Figure 2: Oxygen dissociation curve of normal human haemoglobin (B) in comparison to the curve of haemoglobin with high oxygen affinity (A) and with low oxygen affinity (C). The normal dissociation curve shifts slightly to the left when blood pH increases and when temperature and the concentration of 2,3-diphosphoglycerate (2,3-DPG) decrease. The opposite deviation occurs when pH decreases or temperature and 2,3-DPG concentration increase. Source: Dimitris L Loukopoulos, 2002.

4. Haemoglobinopathies

The above paragraphs emphasize that oxygenation of red cells is performed by a very complex mechanism where the main important points are: (a) the adequate production of red cells, (b) a satisfactory complement of the latter with hemoglobin molecules (300106 or 30pg per erythrocyte), and (c) a very precise structure of the respective globin chains. The conditions where these three important points are not met, then there is an abnormality in the haemoglobin which is known as haemoglobinopathies. Thalasaemia is termed when the conditions is completely or partially inhibit the rate of synthesis whereas variants are termed when there is a change in the structure of the primary globin chain.

4.1. Thalassaemia

Thalasaemia represents a group of hereditary disorders of haemoglobin synthesis with varying severity. The production of normal haemoglobin (HbA) is inhibited due to anomaly in the orderly synthesis of one or the other peptide chains of the haemoglobin molecule. They differ, however, from the other

disorders of haemoglobin formation in that no abnormal haemoglobin chains are formed. Rather, the rate of adult haemoglobin formation is diminished and as a consequence, various combinations of normal peptide chains may exist in abnormal quantity. The thalassaemias result from inherited defects in the synthesis of the globin chains of haemoglobin. Humans have four haemoglobins at different stages of development. All the four different haemoglobins are tetramers of two pairs of unlike globin chains. Every individual globin chain has a haem moiety attached to it, to which oxygen is bound.

With the advance in the knowledge of the structure of the haemoglobin molecule, it is now possible to distinguish a great majority of thalasaemias. The two commonly found thalasaemias are β -thalasaemia in which β chain synthesis is reduced and α-thalasaemia where α chain synthesis is affected. Both α and β thalasaemias may exist either in the heterozygous state (when the gene for thalasaemia is inherited from either father or mother) or in the homozygous state (when the gene is inherited from both parents).

α Thalassaemia

A normal humans receive α genes from each parents (αα/αα), so genetically its is very complicated. There are two main classes of α thalassaemia. One is α^0 thalassaemias where both α genes are deleted; i.e., all or part of the gene is missing. The homozygous state is written --/ --, and the heterozygous state is written --/αα. The other one is α^+ thalassaemias where only one of α genes is lost; the homozygous and heterozygous states are designated as –α/αα and -α/-α, respectively. Sometimes α^+ thalassaemia results from a mutation that inactivates α globin gene rather than deleting it. In this case the heterozygous state is written α^Tα/αα.

β Thalassaemia

180 or more different mutations of the β globin genes are found in patients with β thalassemia. They may affect gene function at any level between transcriptions, processing of the primary messenger ribonucleic acid transcript, translation, or post-translational stability of the gene product (Encyclopedia of Genetics, 2003). Rarely, β thalassaemia, like α thalassaemia, may result from a partial or complete deletion of the β globin gene. Some of these mutations cause an absence of β-chain production and the resulting disease is called β^0 thalassaemia, whereas others result in a reduced output of β chain, β^+ thalassaemia. Some of the latter forms are extremely mild and may not identifiable in carriers; most hetrozygotes for β thalassaemia have very mild anaemia and a raised level of HbA_2.

The hallmark of all the thalassaemias is imbalanced globin chain production. In the β thalassaemias this results in an excess of a chains, which precipitate in the red cell precursors, leading to their damage in the bone marrow and shortening the survival of their progeny in the peripheral blood. The pathology of α thalassaemias is different. In the face of defective α-chain production excess γ chains produced in fetal life form γ_4 molecules, while in adults excess β chains form β_4 molecules; these homo tetramers

are called Hbs Bart's (γ_4) and H (β_4) respectively. They do not give up oxygen affinity of the homo tetramers leads to reduced oxygen delivery to the tissues (Weatherall, 2001).

4.2. Haemoglobin Variants

Each nucleotide across α and β gene sequences are expected to have a chance of mutation. However, several mutations (especially those of the third nucleotide of the respective triplet) do not result in amino acid replacement, because the change still codes for the same amino acid. In fact, of the 2600 possible mutations of the α and β genes, only 1690 are expected to result in an amino acid substitution and, of these, only about one-third have so far been identified (approximately 200 α-chain and more than 300 β-chain variants) (Huisman et al., 1996); this number is continuously increasing. Haemoglobin variants that are used earlier to characterize with the capital has been shifted today to the correct annotation which gives the type of chain, the number of the amino acid in the helical or non helical sequence, and the abbreviated amino acid change. For example, hemoglobin S is referred to as β6 (A6) glutamic acid-valine.

Some variants are sporadic because in many cases, it is not possible to identify the mutation even in the parents. Contrast to it, some variants occur more frequently but independently, reflecting a 'hot' mutational 'spot'; an example is the mutation of leucine to proline or arginine, which results from a change of the nucleotide thymine (position 2 in the respective triplet) to cytosine or guanine.

Endemic hemoglobin variants

(a) Hemoglobin S: β6 (A3) glutamic acid to valine

This is the most commonly found haemoglobin variant mostly found among the black people but also found around the Mediterranean Sea and in India. Sickle cell haemoglobin (HbS) is a molecular abnormality of beta globin chain with the substitution of Glutamic acid by valine at 6^{th} codon position. This particular mutation grossly affects the solubility and crystallization of this haemoglobin under conditions of hypoxia. Sickle cell disease is an autosomal hereditary disorder where the red cells become a sickle shape and this disease resulting from homozygous condition is a haemolytic anaemia. The disease is characterized by enlarged spleen, painful crises, organ damage, impaired mental functions, increased susceptibility to infection and ultimately early death under certain conditions.

(b) Hemoglobin C: β6 (A3) glutamic acid to lysine

This haemoglobin variant is also common among the black people in West Central Africa. This variant resulting from homozygosity will lead to decreased solubility, target-shaped red cells, and haemolytic anaemia as it is also in HbS.

(c) Hemoglobin E: β26 (B8) glutamic acid to lysine

This variant is mostly found in the Far East, where the frequency of heterozygotes range from 10% to 25% (mainly Thai and Chinese, but not Japanese or Polynesian populations). The variant chain is associated with mild instability and a slightly reduced rate of synthesis (through the abnormal additional activation of a 'cryptic' splicing site and wasting of some β^E mRNA). As a result, homozygosity for the variant causes mild haemolytic anaemia, but it becomes very severe with compound β^E/β-thalassaemia heterozygotes.

(d) Hemoglobin D_{Punjab}: β121 (GH4) glutamic acid to glutamine

This haemoglobin D variant is found in India, and was first reported from Pune in a Sikh soldier (Bird et al 1955). Later further investigations in Punjab showed further incidence of HbD in Sikh and Punjabis (Bird et al 1956), and also some parts of India such as Uttar Pradesh, West Bengal, Assam, Gujarat, Mumbai, Goa, Tamil Nadu, Kolkata, Kerala (Basu et al. 1994). It is not usually associated with any clinical manifestations.

(e) Hemoglobin O_{Arab}: β121 (GH4) glutamic acid to lysine

This haemoglobin variant is found among the Pomacs, probably representing an ancient Thracian tribe in Greece and Bulgaria. Its homozygous state is associated with mild hemolytic anemia, which is aggravated in compound b^0/b-thalassaemia heterozygotes.

Sporadic hemoglobin variants

The most common examples of sporadic haemoglobin variants are Hemoglobin with increased oxygen affinity, Hemoglobin with decreased oxygen affinity, Haemoglobin M, Unstable haemoglobin, Hemoglobin with more than one mutation in one chain, 'Hybrid' hemoglobin, Thalassaemic haemoglobinopathies, Hemoglobin with elongated chains, and Hemoglobin with shortened chains.

5. Identification of Haemoglobin Variants

Haematological parameters

The reason why majority of the haemoglobin variant display normal in red cell indices is that the amount of the variant haemoglobin synthesized in the red cells of a heterozygote is expected to be equal with the HbA. Many patients with sickle cell anaemia (HbS) are in reasonably good health most of the time and achieving a steady state level of fitness. The importance of early recognition and subsequent clinical and haematological assessement of the disease are greatly facilitated by familiarity with the patient's steady state. A patient with sickle cell anaemia is said to be in steady state when there is absence of infection, acute complicating factors or acute clinical symptoms or crisis for at least three months (Bookchin and Law, 1996).

The levels of total haemoglobin have no way to change, except in some conditions such as mutations that alter the oxygen affinity of the variant and result in anemia or polycythaemia. Of course, increased

numbers of reticulocytes, lactate dehydrogenase and indirect bilirubin levels indicate haemolysis, while the detection of Heinz bodies in red cells points to a promptly precipitating Hb variant. In other cases, the presence of the variant is indicated by other specific properties such as abnormal polymerization and low solubility, which produce distinct alterations in red cell morphology.

Biochemical findings

The main approach of identifying the Hb variant lies in its demonstration and quantification by biochemical studies such as Electrophoresis, Chromatography, Spectral analysis, Oxygen affinity, Biosynthesis, Solubility and thermal stability, Peptide analysis, and DNA (molecular) studies are used in Identification of Haemoglobin Variants.

6. Co-inheritance of Thalassaemia with Haemoglobin Variants

Most of the haemoglobin variants are rare, except there are three which are found very common they are haemoglobin S, C and E. Hence, it is common for a person with β thalassaemia to co-inherit a gene for one of these variants (Weatherall, 2001). The compound heterozygous state for β thalassaemia and the sickle cell gene, sickle cell β thalassaemia, results in a clinical picture very like sickle cell anaemia. On the other hand, the inheritance of β thalassaemia together with haemoglobin E, a haemoglobin variant that is produced at a reduced rate and hence is associated with a mild β thalassaemia phenotype, produces a severe form of thalassaemia which is usually, but not always, transfusion dependent. Haemoglobin E β thalassaemia is one of the commonest forms of severe thalassaemia, and is assuming a major public health problem in parts of India, and further east, particularly in Thailand and Indonesia (Weatherall, 2001).

7. Summary

Broadly, the term haemoglobinopathies circumscribe a large group of inherited disorders involving the amount or structure of hemoglobin molecules, which are contained in the red cells of humans and other higher species. Precisely, the term applies mainly to the structurally abnormal globin chains, which result in variant hemoglobin's with mild or severe, consequences for the carrier. The haemoglobinopathies may occur in high frequency with a varying clinical and social impact in various populations over the globe, or they occur as rare events, some of which represent unique experiments of nature and give us clues to the understanding of haemoglobin function.