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HEMOGLOBIN SYNTHESIS : HEME SYNTHESIS, GLOBIN SYNTHESIS, HEMOGLOBIN TESTING, PORPHYRIA AND THALASSEMIA

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ABSTRACT

As part of the development of a model for the study of hemoglobin synthesis, we have investigated heme synthesis, globin chain production, hemoglobin testing, porphyria and thalassemia. Hemoglobin synthesis is a fundamental biological process essential for oxygen transport, which involves the coordinated production of heme and globin protein chains within erythroid precursor cells in bone marrow. A systematic search for any type of report published and unpublished, was made to review the evidence that hemoglobinopathies occur by deletion and mutation of chromosome 16 and chromosome 11 as well as ineffective enzymes in heme synthesis. Data from the Ebril Thalassemia Center reported 963 cases of hemoglobinopathies by end of 2020, includes 758 cases of thalassemia major, 84 cases of thalassemia intermedia, 60 cases of sickle cell diseases, 37 cases of sickle cell trait and 24 cases of other variants. The prevalence increased from 31.9 per 100,000 individuals in 2015 to 42.7 per 100,000 populations in 2020. Hemoglobin synthesis research has led to a deeper understanding of human body physiology, pathology, and molecular medicine with advancements in oxygen transport, genetic disease and improving potential therapies. This activity outlines the understanding of the molecular mechanisms of hemoglobin, which has been pivotal in advancing therapeutic interventions like hydroxyurea and gene therapy, where it is indicated as part of the interprofessional team and improving patient outcomes from anemia.

INTRODUCTION

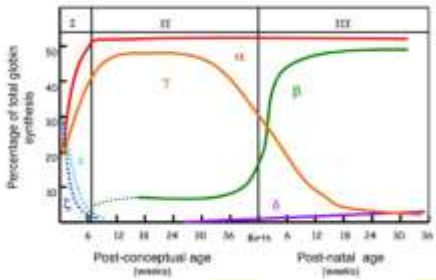
Hemoglobin is a complex tetrameric iron-containing protein compound present in erythrocytes and responsible for oxygen and carbon dioxide transport. Hemoglobin consists of two groups i.e. heme + globin. Each hemoglobin molecule contains four polypeptide globin chains and each globin subunit contains a heme group formed of organic protoporphyrin ring and iron ion in ferrous state, present in the center of the ring. These two components of hemoglobin synthesis are globin production and heme synthesis. The process of hemoglobin synthesis occurs during the maturation of red blood cells within the RBC organelles. The matured RBC doesn't have cell organelles which are essential for synthesis of heme group as well as synthesis of polypeptide globin chains. Most common type of hemoglobin chain in adults is HbA, ultimately making upto 95% - 98%.

The hemoglobin synthesis is mainly synthesized in an erythrocyte producing cell of bone marrow known as erythroid precursors with the help of several enzymes and nutrients like iron and pyridoxine. The hemoglobin synthesis involves the coordinated gene expression, iron metabolism and heme biosynthesis. Where heme is a prosthetic group of hemoglobin that occurs partially in the mitochondria and partially in cytoplasm (both are required). The heme synthesis begins with glycine and succinyl CoA and ends with the formation of a protoporphyrin IX ring, which binds with iron ions. Same as the globin chain synthesis takes place in the cytosol of erythrocytes precursors in

ribosomes by globin gene expression and their regulation, where the coordinated expression of α-globin genes and β-globin genes are mandatory. If uncoordinated expression should occur in any gene can cause the formation of abnormal hemoglobin or hemoglobinopathies that reduces the oxygen carrying capacity or earlier hemolysis of hemoglobin by liver and spleen.

FUNDAMENTALS

There are different forms of normal hemoglobin found in human red blood cells. Normal forms of hemoglobin present prevalence based on the stage of human development. At the different stages of human development found different forms of hemoglobin formation. Commonly, during the pregnancy, there are two forms of hemoglobin synthesized. First is embryonic hemoglobin i.e.Gower 1 (ζ2ε2), Gower 2 (α2ε2), Portland (ζ2γ2), which is produced in yolk sac between first 6-8 weeks of gestation and later replaced by fetal hemoglobin (HbF). These embryonic hemoglobin have a high affinity of oxygen that are necessary for embryonic development. And the second is fetal hemoglobin (HbF,α2γ2)which is composed of 2 alpha chains and 2 gamma chains. The fetal hemoglobin produced in the liver and spleen after that bone marrow takes place. This fetal hemoglobin (HbF) persistence approx 70%-90% at birth and gradually replaced by HbA. HbF has higher oxygen affinity as compared to adult hemoglobin (HbA), facilitating oxygen to flow from maternal to fetal circulation via placenta. HbF production drops significantly after the birth and reaches low, near adult level at the age of 2 years. The fetal hemoglobin may also present in small amounts <1% in adults but increases its level when conditions like sickle cell anemia or beta-thalassemia should occur.



Adult hemoglobin (HbA, α2β2) is the most common form of hemoglobin, which replaces the HbF after birth. The HbA is composed of 2 α-globin and 2 β-globin subunits and primarily produced in bone marrow. HbA ultimately makes up 95%-98% of hemoglobin in adults and efficiently transport and release oxygen within the body. Another hemoglobin A2 (HbA2, α2δ2) is less common adult form of hemoglobin composed of 2 alpha (α) chains and 2 delta (δ) chains subunits. It makes up 1%-3% of hemoglobin in adults, which production increases in beta-thalassemia and decreases in iron deficiency anemia conditions. Each type of hemoglobin plays an essential role in oxygen transportation and production of variants can lead to different types of hemoglobinopathies.

HEME SYNTHESIS

Heme is an iron-containing porphyrin compound which is mainly synthesized in erythroid precursor cells of the bone marrow and in the liver. The chemical formula of heme is C34H32FeN4O4 and its molecular weight is about ~616 g/mol. The heme synthesis is accomplished by eight enzymes, 4 working in the mitochondria and 4 in the cytosol. So, the mature RBC doesn't make hemoglobin due to lack of mitochondria. Synthesis of heme involved mainly five steps, these are formation of δ-aminolevulinate (ALA), synthesis of porphobilinogen (PBG), formation of porphyrin ring, conversion of uroporphyrinogen III to protoporphyrin IX and synthesis of heme from protoporphyrin IX. The process of heme synthesis starts with the combination of succinyl coenzyme A (intermediate in krebs cycle) with glycine (non-essential amino acid) and ends with the formation of heme group. These steps are described below :-

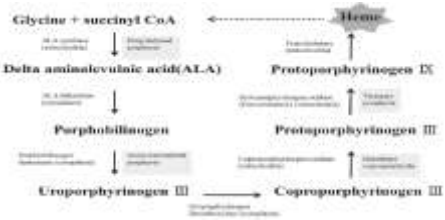
1.Formation of δ-aminolevulinate (ALA) : The process of heme synthesis is started with the formation of ALA in mitochondria, where Succinyl CoA links with Glycine in the presence of ALA synthase . In this process CoA, sulphur, hydrogen and carbon dioxide molecules has been removed. It is catalysed by pyridoxal phosphate dependent ALA synthase and regulated by negative feedback mechanisms.

2.Synthesis of porphobilinogen (PBG) : This second process occurs in the cytosol, when two ALA molecules combine and produce porphobilinogen in the presence of ALA dehydratase enzyme and removal of 2H2O (water) is occurs. This ALA dehydratase is sensitive to inhibition by lead.

3.Formation of porphyrin ring : This step is also occurs in cytosol by condensation four molecules of PBG to produce uroporphyrinogen III with the help of uroporphyrinogen I synthase and uroporphyrinogen III cosynthase, where ring closure occurs. In this process 4NH3 is removed and the compound is ready for further process.

4.Conversion of uroporphyrinogen III to protoporphyrin IX : In this process, uroporphyrinogen III converts into coproporphyrinogen III via uroporphyrinogen decarboxylase enzyme in cytosol. Later the further process occurs in mitochondria where coproporphyrinogen III converts into protoporphyrinogen IX with the help of coproporphyrinogen oxidase enzyme. At this time, carbon dioxide is excreted in big amounts. After that protoporphyrinogen oxidase converts protoporphyrinogen IX to protoporphyrin IX with 4H+ removal.

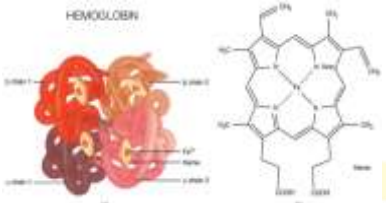
5.Synthesis of heme from protoporphyrin IX : This is the final step of heme synthesis is the insertion of iron (ferrous state) into protoporphyrin IX by ferrochelatase enzyme to produce a heme molecule. This last step occurs in mitochondria where heme synthesis is completed.



The heme synthesis is regulated by the negative feedback by heme in which inhibits ALA synthase and regulates globin chain synthesis for ensuring balanced hemoglobin production. Like that its also regulated by availability of iron and erythropoietin which increases heme and globin synthesis in response to hypoxia and maintains the hemoglobin level in the body. Heme synthesis is a complex and highly regulated biochemical pathway essential for cellular respiration and can be inhibited by lead poisoning.

GLOBIN SYNTHESIS

The globin part of hemoglobin consists of 4 polypeptide chains namely α1,α2,β1 and β2. The alpha chain contains 141×2 = 282 numbers of amino acids and the beta chain contains 146×2 = 292 numbers of amino acids. There are a total of 574 amino acids present in the adult globin chain. Balanced globin chain synthesis is essential for proper hemoglobin functioning because imbalanced synthesis of globin chain leads to hemoglobinopathies like thalassemia, sickle cell anemia etc. The globin chain synthesis begins in the nucleus of the erythroid precursor, where DNA sequences encoding genes are transcribed. This is regulated by globin genes located on chromosomes 16 and chromosomes 11. The chromosome 16 makes up the alpha (α) globin chain, whereas chromosome 11 makes up the beta (β)-like globin chain where they make their clones. Their gene expression is tightly regulated throughout development, transitioning from embryonic to fetal and then to adult hemoglobin, where they make different types of globin chain having variable oxygen affinity capacity. These expressions are regulated by locus control region (LCR) and other transcription factors like GATA-1, KLF-1 and BCL11A that dictate developmental switch in hemoglobin types.



Globin chain synthesis begins with transcription of globin genes, which is followed by mRNA processing, translation on polyribosomes in erythroid precursor cells and post-translational modification. The transcription of globin genes from chromosomes 16, where it makes α-globin clusters (HbA, HbA2) and chromosomes 11, where it makes β-like globin clusters (HbB, HbG, HbD, HbE). After the transcription of genes, mRNA processing starts and pre-mRNA undergoes post-transcriptional modification. Where 5' capping i.e. protects mRNA from degradation and non-coding regions (introns) are removed from pre-mRNA transcript and the remaining coding regions (exons) are joined together to form a mature mRNA molecule, which is ready for translation. Now, the mature globin mRNA is transported to cytoplasm for translation in polyribosomes. The ribosomes assemble globin polypeptide, which fold into functional tertiary structure through the help of chaperone proteins. Normal hemoglobin function α and β globin chain produced in 1:1 ration. Excess impaired globin chains are unstable and undergoes degradation via ubiquitin-proteasome system and completed the translation process. After the translation, globin chains undergo post-translational modification by ferrochelatase insertion of iron (Fe++) into protoporphyrin IX to form heme and binds to globin. Hereby two α-globin chains and two β-globin (or gamma/ delta) chains combine to form hemoglobin molecules. The regulations of globin chain synthesis via heme regulation and erythropoietin(EPO) hormone, where high heme levels promote globin translation and low level inhibit protein synthesis as well as EPO stimulates erythroid precursor and enhancing globin production during hypoxia.

TESTING

Hemoglobin concentration measurement is essential for monitoring and diagnosing patient conditions like anemia, polycythemia and hemoglobinopathies, which is foundational in clinical diagnostic and haematological research. Hemoglobin measurement is a parameter in complete blood count (CBC), which reflects the oxygen carrying capacity of blood. Hemoglobin concentration is measured by Sahil's methods, cyanmethemoglobin methods (Drabkin's method), hematology analyzer or point of care (POC) devices, pulse co-oximetry, mass spectrometry and high performance liquid chromatography (HPLC), Near-infrared spectroscopy (NIRS) etc. Patients with abnormal hemoglobin concentrations have signs and symptoms of hemolytic anemia like increases in unconjugated bilirubin, fatigue, jaundice or hemoglobinuria. CBC is most commonly used for testing of hemoglobin concentration but family history of hemoglobinopathy requires the further testing screens for and diagnosing haematological disorders. Normal hemoglobin concentrations are approximately 13.8 to 18 g/dl in men, 11.5 to 16 g/dl in women, 11.0 g/dl in pregnancy, 11.0 to 16.0 g/dl in children and having highest hemoglobin concentration in newborn baby i.e. 14 to 20 g/dl. CBC also measures the site of erythrocytes through the mean corpuscular volume (MCV), where low MCV is the first indicator of thalassemia and thalassemia traits.

Hemoglobin variant testing measures by percentage of hemoglobin types, which present in erythrocytes. Hemoglobin derivatives and variants have their own morphological significances and pigmentation like oxyhemoglobin having bright red, deoxyhemoglobin having dark red, methemoglobin having brownish, carboxyhemoglobin havin cherry red, sulfhemoglobin having greenish, biliverdin having green and hemosiderin having brownish pigmentation. This pigmentation can help differentiate between hemoglobin variants. The hemoglobin variants like

HbS, HbC, HbD and sickle cell can be identified by hemoglobin electrophoresis, HPLC and capillary electrophoresis methods. Hemoglobin variants testing allow for the detection of hemoglobin variant types and thalassemic disorders, which is helpful for anemia treatment and improving patient outcomes. DNA and genetic testing help identify deletion or mutation in the alpha globin chain and beta globin chain producing gene, which is standard part of newborn screening to improve early detection and treatment of hemoglobin variants (if possible).

PORPHYRIAS

The porphyrias are a group of hereditary or acquired disorders caused by defective heme synthesis. Ineffective enzymes in the heme synthesis pathways result in accumulation of molecules and build of potentially toxic heme precursors. There are six different types of porphyrias that can occur during heme synthesis i.e. Acute intermittent porphyria, congenital erythropoietic porphyria, porphyria cutanea tarda, hereditary coproporphyria, variegate porphyria and protoporphyria. These porphyrias are classified into two categories, one is erythropoietic porphyria (enzyme deficiency occurs in erythrocytes) and another is hepatic porphyria (enzyme defect lies in liver).

1.Acute Intermittent Porphyria (AIP) : AIP is the second most common porphyria after porphyria cutanea tarda (PCT). It is caused by a defect in uroporphyrinogen I synthase that leads to the accumulation of neurotoxic metabolites including ALA and PBG. Caloric deprivation, medication that induce cytochrome P-450 and hepatic ALA synthase precipitate AIP. Its most common finding is urine gets darkened on exposure to air due to conversion of porphobilinogen into porphobilin and porphyrins. The clinical manifestations are acute abdominal pain (upto 90% of patients), constipation, vomiting, cardiovascular abnormalities and neuropsychiatric disturbances. AIP can be treated by hematin, which inhibits enzymes ALA synthase and accumulation of PBG.

2.Congenital Erythropoietic Porphyria (CEP) : This is erythropoietic porphyria caused by defects in uroporphyrinogen III synthase. Main finding in this porphyria is urinary excretion of uroporphyrinogen I and coproporphyrinogen I, which oxidise to uroporphyrin I and coproporphyrin I respectively, leading to urine dark red in colour. Clinical manifestation is photosensitivity (itching and burning of skin when exposed to light) due to abnormal porphyrins that have accumulated and increased hemolysis is also seen in these patients.

3.Porphyria Cutanea Tarda (PCT) : PCT is a most common chronic hepatic porphyria caused by defects in uroporphyrinogen carboxylase (UROD), leads the accumulation of porphyrinogens (such as uroporphyrinogen III) within hepatocytes. Clinically, cutaneous photosensitivity and hyperpigmentation are characteristics of PCT. The gradual formation of vesicles, bullae, blisters, and sores occurs in sun-exposed areas, especially the face and hands. PCT commonly associated with excessive alcohol consumption, hepatitis C, HIV, iron-overloaded. Lever exhibits fluorescence due to high concentration of accumulated porphyrins. The PCT is mostly treated with hematin (inhibits ALA synthase).

4.Hereditary Coproporphyria (HCP) : HCP is hepatic porphyria caused by defects in coproporphyrinogen oxidase. Findings with HCP is coproporphyrinogen III and other intermediate of heme synthesis prior are blockade (eg: ALA, PBG) are excreted in urine and feces (i.e. urinary ALA, PBG, coproporphyrinogen III, and fecal coproporphyrinogen III). Clinical manifestations are photosensitivity, acute abdominal pain, constipation, vomiting, neuropsychiatric disturbances etc.

5.Variegate Porphyria (VP) : It is a rare, inherited metabolic disordered hepatic porphyria caused by defects in protoporphyrinogen oxidase enzymes. Findings with VP are urinary and fecal ALA, PBG, protoporphyrin III, uroporphyrin and protoporphyrin. Clinical manifestation with variegate porphyria is photosensitivity, neuropathic pain, autoimmune instability, peripheral neuropathy due to metabolic buildup and having neurotoxic effects.

6.Protoporphyria : Protoporphyria is a erythropoietic porphyria caused by defects in ferrochelatase enzymes, which insert iron into protoporphyrin IX. Findings with protoporphyria is protoporphyrin IX accumulates in tissues that causes acute painful photosensitivity and potential liver diseases, which is excreted in urine and feces. This porphyria typically presents in early childhood with immediate pain upon exposure to bright sunlight. Reticulocytes and skin biopsy exhibit red fluorescence with protoporphyria patients.

Porphyria can be diagnosed by detecting elevated liver of porphyrins and its precursors in urine or blood. It can also be diagnosed by using UV fluorescence technique to detect porphyrins and PBG in urine using Watson-Schwartz test. Study of porphyria is essential for diagnosing and treating heme synthesis disorders and improving the patient outcomes.

THALASSEMIA

Thalassemia are hereditary disorders caused by reduction or absence of one or more of globin chain that make up hemoglobin. Thalassemia results from deletion or mutation in genes encoding either alpha (α) or beta (β) globin chains. Deletion or mutation in HBA1 or HBA2 genes on chromosomes 16 impair the production of α-globin chains, leading to excess unpaired β or γ chain, which forms abnormal hemoglobin variants like HbH (β4) or Hb Bart's (γ4) leading the α-thalassemia. β-thalassemia caused by deletion or mutation in HBB gene on chromosomes 11, results in reduced (B+) or absence of (B0) of β-globin production leading to an accumulation of unpaired α-globin chains that precipitate in erythroid precursors leading to the oxidative damage, hemolysis and bone marrow expansion.

Alpha thalassemia comprises four subtypes depend on severity of the anemia, which all are caused by α-globin gene deletion that negatively impacts alpha globin subunit synthesis. The difference in subtype is the number of alpha globin gene deletion. One gene

deletion results in α-thalassemia (known as α-thalassemia minima), which has no significant haematological consequences. Two gene deletion results in α-thalassemia (α-thalassemia minor), causes mild microcytic, hypochromic anemia. It is also known as cis deletion, where two gene deletion on the same chromosome, are prevalent in the Asian population. Where one deletion in each chromosome 16 known as trans deletion, which is prevalent in African American populations. Three gene deletion results in hemoglobin H (HbH) diseases. HbH is unstable form of hemoglobin that precipitate and causes damage to erythrocytes as they age. Four gene deletion result in hemoglobin Bart's disease (Hb Bart's), which is incompatible with life. The absence of α-globin subunits allow γ-globin subunit in utero to combine and form gamma (γ) tetramers. Hb Bart's diseases has high oxygen affinity but doesn't allow the release of oxygen to body tissues, which leads to severe hypoxia of the infants known as hydro fetalis. These deletion or mutation are passed down from parents to their children.

Beta thalassemia comprises two major subtypes based on the severity of the diseases. β-globin gene deletion or mutation that negatively impacts β-globin subunit synthesis. Heterozygotes with only one gene mutation have β-thalassemia minor, causes diminished production of β-globin subunit and patient may develop mild microcytic anemia but there is no evidence of hemolysis. Homozygotes with two gene mutation have β-thalassemia major, which causes absent production of β-globin subunit. Lack of β-globin, results in the accumulation of α-globin subunit and forms α tetramers, which damages red blood cells. It leads ineffective erythropoiesis and extravascular hemolysis that causes severe microcytic, hypochromic anemia. In this condition patient may requires chronic blood transfusion.

Thalassemia ranging from mild anemia to severe transfusion dependent anemia, growth retardation, skeletal deformities and iron-overloaded due to frequent blood transfusions. Thalassemia is prevalent in malaria endemic region such as the Mediterranean, southeast Asia and part of Africa, due to protective effects against plasmodium infection in carriers. Thalassemia can be diagnosed by CBC, hemoglobin electrophoresis and genetic testing. Patient with thalassemia can be managed by regular blood transfusions, iron chelation therapy, folic acid supplementation and bone marrow transplantation. Some research on hemoglobin synthesis involves the gene editing (eg: CRISPR-Cas9 targeting BC111A) and fetal hemoglobin synthesis reactivation emerging therapies may treat the thalassemia in future.

CONCLUSION

Hemoglobin synthesis is complex and tightly regulated process involving the coordinated production of heme and globin chain within erythroid precursor cells. It requires precise transcriptional and translational control to maintain a balanced α:β globin chain ratio. There are different types of globin chain formation takes place at different developmental stages like embryonic hemoglobin (Hb Gower 1, Gower 2, Portland), fetal hemoglobin (HbF), adult hemoglobin (HbA), which is regulated by globin gene expression. Disruption in any step of globin synthesis by deletion or mutation in chromosome 16 and chromosome 11 can lead to severe hemoglobinopathies like thalassemia and sickle cell anemia, which are ineffective to transport sufficient oxygen to the body cells. The heme synthesis takes place in cytosol and mitochondria of premature erythrocytes. Heme synthesis accomplished by eight enzymes and five steps. Ineffective enzymes in heme synthesis cause defective heme formation and can lead to different types of porphyria, which has reduced capacity for gas transportation within the body. So, coordinated production of globin chain and heme synthesis is essential for normal hemoglobin synthesis, which is able to transport sufficient oxygen and overall erythrocyte functions. Understanding of hemoglobin synthesis helps the interprofessional team to diagnose and open new avenues for personalized and potentially curative treatment for hemoglobinopathies and improving patient outcomes from anemia.

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