Genetics and Epigenetics Aspects of Thalassemia

Inayu Mahardhika Putri[1], Ferry P. Gultom[2(✉)], and Elza Ibrahim Auerkari[2(✉)]

[1] Pedodontic Specialist Student, Faculty of Dentistry, Universitas Indonesia, Jalan Salemba Raya No. 4, Central Jakarta 10430, Indonesia
[2] Oral Biology Lecturer, Faculty of Dentistry, Universitas Indonesia, Jalan Salemba Raya No 4, Central Jakarta 10430, Indonesia
gultom_ferry@yahoo.com, elza.ibrahim@ui.ac.id

Abstract. Hemoglobin contains of heme, which binds iron and protoporphyrin, and globin, which consist of combination of α, β, δ, and γ chain. α-globin globin gene is located in 16p13.3 cytogenetically, and β-globin gene is in 11p15.4. Thalassemia is a blood disorder characterized in the lack or absence of one of globin chain. Thalassemia is classified as alpha thalassemia and beta thalassemia depending on which globin chain is reduced. Alpha thalassemia occurs when alpha globin chain in chromosome 16 is reduced or absence due to removal or deletion in the molecular level. Alpha thalassemia itself has several types including alpha thalassemia silence carrier, alpha thalassemia minor, haemoglobin H disease, and Hydrops fetalis. Alpha thalassemia silence carrier and alpha thalassemia minor is clinically asymptomatic. Hemoglobin H disease shows moderate to severe anemia symptom, while Hydrops fetalis is fatal. The reduced or absence of alpha globin chain causing the excess of beta globin chain. Beta thalassemia occur when beta globin chain synthesis is compromised mainly because of point mutation in chromosome 11. Beta thalassemia is divided into beta thalassemia minor, intermedia, and major. Beta thalassemia minor is clinically asymptomatic, while intermedia type shows symptoms between the minor and the major. Beta thalassemia major is the most severe among beta thalassemia, and regular blood transfusion may be needed of the anemia symptoms are increased. Besides genetic factors, epigenetic factors also play roles in causing thalassemia. Treatment of thalassemia is depended on its symptoms, such as blood transfusion and surgical treatment. Genetic therapy for thalassemia is still in development of therapy options.

Keywords: Thalassemia · Genetics · Epigenetics

1 Introduction

One of the most common forms of anemia worldwide is thalassemia. Thalassemia is an inhereditary anemia. It characterizes the disorder in the synthetis of hemoglobin chain. Imbalaced product of hemoglobin chain cause the decrease of red blood cells and give the anemic appearance such as pale. Besides that Thalassemia is an inherited blood disorder that causes your body to have less hemoglobin than normal. Hemoglobin enables red blood cells to carry oxygen can cause anemia and fatigue [1]. Thalassemia is divided into two types depending on which specific hemoglobin chain is imbalanced. There are

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alpha thalassemia and beta thalassemia. Each type divided into several subtypes with their own severity. The most severe type of alpha thalassemia is Hydrops fetalis which is lethal in fetus, while in beta thalassemia is thalassemia major.

Thalassemia was first recognized in 1925 in United States of America and Italy by Dr. Thomas Cooley. It was first called as Cooley's anemia. Then it changed into thalassemia which contains of two Greek terms, Thalassa (sea) and Emia (blood). Thus thalassemia was anemia happens in Mediterranean. Nowadays Cooley's anemia is also known as thalassemia major. Thalassemia major is the severe form of beta thalassemia. Thalassemia do not happen only to the Mediterranean area now, but also in Africa, Middle East, and Southeast Asia [1, 2].

In Indonesia, specifically in the city of Yogjakarta, a research shown that the prevalence of people with thalassemia carrier is almost 45% amongst the samples. it showed a significantly high percentage. Since thalassemia is an autosomal recessive disorder, there is 25% chance of having thalassemia if both parents are carriers. It is important to have knowledge in this disease since thalassemia carriers are quite common [3]. Some factors are involved in causing thalassemia including genetics and epigenetics. This script will depict some of the genetics and epigenetics side of thalassemia.

2 Literature Review

2.1 Structure of Hemoglobin

Hemoglobin was in tetramer form contains of heme as a non-protein substance and globin as a protein substance. Heme contains iron and protoporphyrin. Globin chain consists of combination of α, β, δ, and γ chain. In adult, adult hemoglobin (HbA) made the most proportion of all hemoglobin by 96–98%, then followed by small quantities of two other hemoglobins (HbF and HbA_2). HbA consists of two pairs of polypeptide chains consist of two β globin chains (β_2) and two α globin chains (α_2) ($\alpha_2\beta_2$). HbF contains of two α globin chains and two γ globin chains ($\alpha_2\gamma_2$). HbA_2 contains of two α globin chains and two δ globin chains ($\alpha_2\delta_2$). HbF is found with the highest percentage during fetal life, then after birth it is then switched into adult hemoglobin ($\alpha_2\beta_2$) [4, 5].

Chromosome 16 is responsible for controlling the synthesis of α globin gene, specifically in the cytogenetic location of 16p13.3, which located in the position of 13.3 in the short (p) arm of chromosome 16. It's molecularly located in base pair 176,651 to 177,522 on chromosome 16, while the synthesis of β globin chain take part in chromosome 11 with the exact location of 11p15.4, meanings it is located in position 15.4 in the short (p) arm of chromosome 11. It is molecularly located in base pair 5,225,466 to 5,227,071 on chromosome 11. There are four elements which regulate the α-locus known as MCSR1 to MCSR4. There are five elements which regulate the β-locus known as locus control region (LCR). During the developmental periods, there are several hemoglobin types expressed. In embryonic development stage Hb Portland ($\zeta_2\gamma_2$), Hb Gower-I ($\zeta_2\varepsilon_2$), and Hb Gower-II ($\alpha_2\varepsilon_2$) are expressed. In fetal stage HbF ($\alpha_2\gamma_2$) are expressed. In adult stage HbA_2 ($\alpha_2\delta_2$) and HbA ($\alpha_2\beta_2$) are expressed. In fetal life, HbF has bigger role, then it switch to HbA after birth [4–6].

2.2 Thalassemia in Heritance

Thalassemia is an autosomal recessive inherited condition. It means that a child would have thalassemia disease if he inherited the mutated thalassemia gene from both parents. If only one mutated gene is inherited, then a child is a carrier thalassemia. Most carriers are asymptomatic and live healthy lives. Both parents are carriers, a child has 25% chance of having two mutated genes and initiating the disease, also 50% chance of becoming a thalassemia trait carrier.

The two types of thalassemia (alpha thalassemia and beta thalassemia) have the manner in inheriting the disease [7].

2.3 Types of Thalasemia

2.3.1 Alpha Thalassemia

Human α-globin cluster (5′-ζ-α2-α1-3′). MPG, NPLR3, and Luc7L are the expressed genes surrounding the cluster. MCSRs are below thzese genes. The regions of duplication are X, Y, and Z boxes that play a part in generating the common α-thalassemia [8]. Alpha thalassemia is mainly caused by a removal/deletion of one or two alpha genes at the molecular level. Alpha globin gene, which located in chromosome 16, consist of two fragments (α1 and α2) positioned sequentially. Every human has two alpha genes in each chromosome 16, or as usually mentioned as 4 copies (αα/αα) [2, 9] α-globin genes are expressed mostly with the role of MCS-R2 known as HS-40. MCSR2 is placed in the region of 25–65 kb upstream of the α-globin genes, and is one of four MCS (Multispecies Conserved Sequences). These four MCSs (MCSR1-MCSR4) know to take part in regulating the α-like globin genes. X, Y, and Z boxes are the region of duplication which embedded the duplicated α-globin genes (αα). $-\alpha^{3.7}$ (a rightward deletion, which are 3.7 kb apart) is a gene produced in chromosome experiencing reciprocal homologous recombination between Z segments. $-\alpha^{4.2}$ (a leftward deletion, which are 4.2 kb apart) is produced during recombination between homologous X boxes [8].

α-gene may be decreased or not synthesized at all and give several different outcomes. The more missing genes, the more severe the outcome may become. The number of genes involved influenced the severity of the outcome. According to the number of α-genes involved, alpha thalassemia is classified into several types [2, 10]:

1. Alpha thalassemia silence carrier

This type is cause by the deletion of one α-gene (-α/αα). Clinically and hematologically is unseen in patient, meaning it is asymptomatic. Patients only have very little possibility of showing anemic features. Red blood cells abnormality in morphology is not found in this type. These conditions will only found by molecular laboratoric findings.

2. Alpha thalassemia minor

This type is caused by the deletion of two α- gene. This type is shown as heterozygous α-thalassemia (--/αα) or homozygous α-thalassemia (-α/-α). This type shows mild anemia findings. Laboratorically, microcytosis and hypochromia is found in this type. At birth, 4–6% of Hb Bart's are present. Clinically it is asymptomatic and does not need transfusion for its management.

3. Hemoglobin H disease

This type is caused by deletion of three α-gene (--/-α). The decreased of α-globin expression causes a moderate anemia with microcytosis, hypochromia and red blood cell fragmentation. The most severe but non fatal form of α-thalassemia syndrome is this type. Only one α-globin gives the supposed function, causing a turbulent imbalance of globin chain synthesis making some surplus β-globin chain synthesis. Some of the phenotype expression of this disease are moderate to severe anemia with hypochromic and microcytic red blood cells. Some of the clinical features are jaundice, splenomegaly, gallstone. Occasionally, blood transfusion is needed as the management of this disease.

4. Hydrops Fetalis

This type is caused by deletion of four α-gene (--/--). The most intense form of α-thalassemia is Hydrops fetalis, and mostly take place in infants whose both parents posses α-thalassemia syndrome. HBF ($\alpha_2\gamma_2$) is not synthetized during the intra uterine period, then it will cause the unpaired γ-globin chain to aggregate forming γ4 known as HB Bart's. Hb H, small amounts of embryonic hemoglobins, and Hb Bart's are the only hemoglobin produced in infants with hydrops fetalis laboratoric findings show hypochromic and microcytic red blood cells. On clinical examination, these infants show severe anemic features, massively enlarged organs, and heart failure.

2.3.1.1 Pathophysiology of Alpha Thalassemia

In α-thalassemia, the major cause of it is the decreased synthesis of α-globin chain. The reduced or absent α-globin chain will then accumulate normal β-globin chain in adults and γ-globin chain in fetus. The excess β-globin chain will then form β-4 tetramers, known as HbH. In fetus, Hb Bart's or γ-4 tetramers (γ4) if formed due to the excess γ-globin chains which accumulate. These tetramers are unable to deliver oxygen to the tissue and made some tissue had decreased oxygen causing anemia. There are four components causing anemia in α-thalassemia: 1) Ineffective erythropoiesis, 2) reduced or absent of α-genes, 3) intracorpuscular haemolysis, 4) production of HB Bart's and HBH because of inadequate numbers of α-globin chain.

2.3.2 Beta Thalassemia

β-thalassemias are most common in the Mediterranean region, Southeast Asia including China, Indonesia, the Middle East, India. Unlike α-globin gene, β-globin gene is not duplicated and located on chromosome 11 with the exact location of 11p15.5. Thus each cell only contain one β-globin gene. β-thalassemia is mainly happened because of point mutation causing the reduction of β-globin gene. Most of the mutation are minor nucleotide substitution within the cluster, but sometimes deletion may also happen and caused thalassemia. These mutation might cause the alleviation in the synthesis of β-globin gene (β^+-thalassemia) or even the absence of the synthesis of β-globin chain (β^0-thalassemia) [10].

Beta thalassemia are defined into 3 types according to the clinical and laboratory findings. These types are also differ in the β-gene globin involved. These types are [2, 10]:

1. Beta thalassemia minor (β/β^+; β/β^o)

These types also known as carrier of thalassemia. The most important test needed to be done to determine if a patient is having this type is the Mentzer Index. It is important to differ this type of thalassemia with iron deficiency anemia. Patients with beta thalassemia have the Mentzer index of <13. If the Mentzer index shows >13, iron deficiency is more common. Hb A_2 is usually increased in beta thalassemia patients. Excess of α-chain are often found. Comparing to iron deficiency anemia, mean corpuscular volume (MCV) are usually lower and red cell count (RBC) are usually higher. There is a 25% risk of having children with homozygous thalassemia when both parents are carriers. Clinically it is asymptomatic. Mild anemia, elevated HB A2 and F, hypochromia and microcytosis are seen in laboratory findings.

2. Beta thalassemia intermedia (β^+/β^+; β^+/β^o)

Patients with this type of thalassemia have Hb of 7 g/dL without transfusion. Clinically, they have symptoms between the utmost of thalassemia minor and major. They might not need or might occasionally need transfusion since they also show mild anemia symptom.

3. Beta Thalassemia Major (β^o/β^o)

This type is also known as Cooley's anemia. It was first found by Thomas Cooley in 1925. Clinical findings are mostly seen between the age of six and twenty four months. These infants may have feeding problems, irritability, diarrhea, expansion of abdomen caused by spleen and liver expansion. Clinically, there are jaundice, pallor, growth retardation, skeletal changes because of the expansion of the bone marrow. Regular blood transfusion is needed if the severity of anemia increased. Some complications may have developed due to blood transfusion such as iron overload which lead to growth retardation and failure or delayed sexual maturation. Laboratory findings, Hb A are decreased of even absent, there are RBC fragments and striking morphologic, and also hypochromia and microcytosis.

Beta thalassemia happened by the reduced production of β-globin gene chains or complete absence of β-globin gene production. For the beta thalassemia which caused by the reduced β-chain production, is generated by mutations in the promoter zone (either the CACCC or TATA box), 5' and 3' UTR (untranslated regions), the polyadenylation signal, and splicing abnormalities. While beta thalassemia cause by the complete absence of β- chain output is resulted from initiation codon, deletion, nonsense, frameshift, and splicing mutation [11].

2.3.2.1 Pathophysiology of Beta Thalassemia

The reduced or complete absence of β-globin chains conducts to imbalance α-/non α-globin chains. α-globin chains are excessive at this time then accumulate to form α-globin tetramers and bend in the erythroid precursors which lead to oxidative membrane breakdown and comprehensive premature demolition by apoptosis of RBC precursors in the bone marrow. Impractical erythropoiesis causing the damage of red blood cells

and hemolysis. Severe anemia is found due to this primary pathology. Bone deformities and osteopenia are also found due to marrow expansion which also lead to iron overload caused by increased iron absorption of gut. Some of the final findings due to iron overload are tissue iron overload that can be seen in iron induced liver disease, endocrine complication, and even death because of untreated cardiomyopathy [1, 11].

2.4 Epigenetic

2.4.1 Epigenetic of Alpha Thalassemia

One of mutation type that common happen in α-thalassemia is deletion of α-thalassemia Southeast Asia (SEA), which is communal in Southeast Asia. Four genes and one pseudogene are deleted in this mutation. Those genes and pseudogenes are θ-globin gene (HBQ1), pseudo α-globin gene (HBAP1), μ-globin gene (HBM), α-globin 1 gene (HBA1), α-globin 2 gene (HBA2).The homozygous configuration of the allele may lead to hydrops fetalis [12].

Lots of studies were held to determine the epigenetics aspects of α-thalassemia. A research studying SEA deletion between placenta and leukocytes. It is shown that DNA methylation level among placenta and leukocytes is diverse, where DNA methylation level in placenta is much lower than the leukocytes. The SEA deletion happened in the length of 19304 bp, from 165397 to 184700, and involved the genes and pseudogenes mentioned above. Single-nucleotide polymorphism marker rs2541677 close to rs537891147 is causing the deletion. Differential DNA methylation pattern among leukocytes and placenta happened because of the major deletion of α-globin gene cluster [12].

2.4.2 Epigenetic of Beta Thalassemia

Several months after birth, HbF is switch into HbA. A study in some Chinese beta thalassemia individuals tried to find the mechanism which cause the delay of fetal to adult Hb change over, which leads to the clinical complexity of the disease. rSNP (regulatory single-nucleotide polymorphism) rs368698783 was the main predictor of clinical complexity by raising HbF levels. This study found that the insignificant allele of the rSNP may cause an impairment of the LYAR-binding (Ly1 antibody reactive) activity, then triggers the impairment of protein arginine methyltransferase 5 (PRMT5) and two suppressive epigenetic regulators DNA methyltransferase 3 alpha (DNMT3A) from the BBG promoters. Demethylation of the promoter CpG sites in erythroid progenitor cells is one of the results, it also cause the enhancement of γ-globin gene expression [13].

Other study, focusing on IGSF4 and its role in globin synthesis. IGSF4 has an important role in globin synthesis. It was reported that methylation in IGFS4 may cause an interruption in the process of globin synthesis by interacting with other genes in the regulation network of globin expression. Previous findings reported that in thalassemia patients, the promoter of IGSF4 was highly methylated. DNMTs are the main enzyme of genome methylation and it also regulate gene expression [14].

2.5 Management of Thalassemia

Patients need to have a full examination and reviewed before having any treatment. If a patient is showing severe anemic features (Hb < 7 g/dl), transfusion may be needed. But even if a patient has Hb > 7g/dl, other factors should be considered. There may be poor growth and facial changes, so the patient may also need transfusion. For most patients with homozygous beta thalassemia, regular long life transfusion may be needed. Transfusion may cause other complication that needed treatment, such as iron overload. When this happen, iron need to be removed by iron chelation therapy to prevent iron toxicity [1, 10, 11].

Surgical treatment such as splenectomy is indicated if there is enlarged spleen compromising movement and breathing. Nowadays stem cell transplantation (SCT) is considered as the exclusive curative method, it has a successful rate of 80%. It also have risk that leads to death in transplant recipients, if the recipient rejects the transplant [1, 2, 15].

In alpha thalassemia, most of these doesn't need therapy or unable to be treated, Hydrops fetalis is not recommended to be treated due to its high risk of failure. Even if the therapy works, most survivors experienced congenital malformations. HBH disease are most likely not to be treated because patients are just fine without any therapies. Examinations and reviews need to be done to determine the need of treatments. Transfusions and splenectomy may be done if it is needed [9].

2.5.1 Genetic Therapy of Thalassemia

In order to assist patients, thalassemia genetic therapy is now being developed. Research is conducted to discover all possibility. B-cell lymphoma (BCL11A) is a variable of development in B-lymphocytes development. It acts as a growth factor. BCL11A bands to GATA-1 and NURD, then the gamma chain is silenced. In adult blood progenitor cells, it utilizes siRNA to change over gamma to beta chain, then the expression is silenced. This results in the increase of hemoglobin F expression. In maturing red blood cells, EKLF.1 plays a major role. It usually adheres to CACCC sequences, increases its expression, changes the gamma chain to beta, and also silences the gamma chain [2].

Direct repeat erythroid definitive (DRED) is made of two components, TR4 and TR2. It attached firmly to the promoter region's alpha and gamma globin, and DR-1. It leads to increase of HbF due to its inhibitory effect using siRNA. Stage selector protein (SSP) is also made of two parts, P22NF-E4 and CP2. It leads to the increased of gamma chain expression and delayed in beta-gamma change over in cell line K562 [2].

3 Conclusion

Thalassemia is one of most prevalence diseases found in Indonesia especially in children. It is a hereditary disease caused by the reduction or absence of synthesized globin chains. Thalassemia is mainly divided into two categories, alpha thalassemia caused by the reduction and absence alpha globin chains while beta thalassemia caused by the reduction or absence beta globin chains.

Genetics and epigenetics aspects play big role in causing thalassemia. Epigenetic is a hereditary change of gene expression influenced by environment without changing DNA sequences. Epigenetic aspect is a massive field to be studied since it almost has no boundaries. It has lots of possibilities. By knowing the mechanisms of some possible epigenetic events, therapies are developed to get the best results with minimum complication.

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