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CRITICAL REVIEW ON THALASSAEMIA: DIAGNOSIS AND TREATMENT

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ABSTRACT

Thalassaemias are inherited blood disorders that are characterized by imbalance in the synthesis of globin chains, leading to decrease in oxygen carrying capacity of haemoglobin, and consequently causing anaemia with resultant impairment of organs' function. Major types are the alpha and beta thalassaemias. Asymptomatic individuals are estimated to be 1 in 100,000 people and about 10,000 children globally, are born with the disease yearly. While a child with alpha thalassaemia major may die intra-uterine or shortly after birth, those with beta-thalassaemia major present within the first two years of life, with severe anaemia. In the developing world, most of the affected individuals are reportedly dead before they reach the age of 20, and while there is increasing knowledge of the disease in some regions, there are some regions with very low awareness. Unfortunately, the prevalence of thalassaemia is still increasing in some regions. This critical review therefore, x-rays thalassaemia on a general perspective, including its types, prevention, diagnosis, intricacies and treatment

Keywords: Thalassaemia, Mutation, Erythropoiesis, Splenomegaly, Hepatosplenomegally

INTRODUCTION

Thalassaemia, also referred to as 'Mediterranean anaemia', is gotten from the Greek words "*Thalassa*" meaning 'Sea' and "*Emia*" meaning 'blood' (Tari *et al*., 2018). It was found in 1925 by a Physician who undertook studies on Italian children with life threatening anaemia, early childhood mortality, and large abdominal organs; occasioned by reduced synthesis of one or more globin chains that are responsible for low oxygen transport through the body (Shirzadfar and Moktari, 2018). It is an inherited disease in which the body makes less haemoglobin responsible for oxygen transport (Shirzadfar and Moktari 2018). In this anomaly, the level of synthesis of alpha (α) or beta (β) haemoglobin chains are lowered, thereby causing too much damage to red blood cells and giving rise to anaemia (Cheesbrough, 2000).

Thalassaemia syndrome is the most common inherited disorder worldwide, in which a mutation interferes with the number of generated protein (Angastiniotis and Lobitz, 2019). In this type of anomaly, there is a mutation in α-globin (HbA1/HbA2) gene and β-globin (HbB) genes, which are commonly inherited in an autosomal recessive manner (Tari *et al*, 2018). When this type of mutation happens, globin chain production becomes imbalanced, causing an impaired erythropoiesis (Angastiniotis and Lobitz, 2019). The extent of the disease burden is majorly dependent on the level at which of the chain becomes imbalanced (Angastiniotis and Lobitz, 2019). Reduction in the expression of one of the two globin chains, leads to accumulation of too much polypeptides encoded by the unaffected gene (Sabath, 2017). This chain imbalance results to abnormal Red Blood Cell (RBC) maturation, thereby causing microcytosis as a characteristic laboratory anomaly (Lecturio medical online library, 2020). Milder forms of thalassaemia do not result to anaemia, but the more severe forms can result to microcytic anaemia, haemolysis, iron loading and transfusion dependence in the most severe cases (Sabath, 2017).

Diagnosis is by blood tests, including but not limited to full blood count, special haemoglobin tests and genetic tests (Sharma *et al*., 2017). At the moment, management of β-thalassaemia major patients is by frequent transfusion of packed red cells and effective chelating therapy (Sharma *et al*., 2017).

The first haematopoietic stem cell transplant (HSCT) in thalassaemia was conducted over thirty years ago and this procedure has become a widely applied treatment for the definitive cure of thalassaemia major (Sharma *et al*., 2017). Over 3000 haematopoietic stem cell transfusions have been carried out globally and still remained the only

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available curative option for thalassaemia major; though it is very expensive (Sharma *et al*., 2017). There is increasing knowledge of and attitude towards thalassaemia in some regions like Iraq, to the extent that premarital counselling is accepted (Ali *et al*., 2018).

Thalassaemia, like sickle cell disease, is an inherited disorder and can be avoided through premarital counselling and genetic testing (Ali *et al*., 2019). While there is increasing knowledge of the disease in some regions, there are some regions with very low awareness. The prevalence of thalassaemia is increasing in some regions (Sharma *et al*., 2017). It has no cure and can be fatal when untreated (Huang *et al*., 2020). The management of thalassaemia is very expensive in that not many people can afford it, but through premarital counselling the disease can be reduced or completely avoided. Hence this review, geared towards awakening consciousness about the disease and its devastating consequences.

EPIDEMOLOGY

The prevalence of thalassaemia carriers is much among the Mediterranean people, Eastern Europe and the South-East Asian regions (Chuncharunee *et al*, 2019). There are about 30 million carriers and approximately 10,000 children born with the condition yearly the world over (Shirzadfar and Mokhtari, 2018). It is also common in the Middle East, India, Central Asia, Southern China, the Far East and countries along the North Coast of Africa and South America (Sharma et al, 2017). Movement of people and inter marriage between different ethnic populations have introduced thalassaemia in almost countries of the world Angastiniotis and Lobitz, 2019). Notwithstanding, correct data on carrier rates in a number of populations are lacking especially in most places expected to have been heavily affected. (Madan et al, 2010). Thalassaemia affects all sexes equally, occurring approximately in 4.4% of every 10,000 live births (Al-zwaini, 2018). In the developing world, most of the affected individuals are reportedly dead before they reach the age of 20 (Kadhim *et al*, 2017). Maldives have the highest incidence of thalassaemia globally with carrier rate of 18% of the population (Sharma et al, 2017). Thailand is estimated to have the prevalence rate of 16% while Iran is estimated to have 5-10% prevalence and 3-8% from Bangladesh, China, India, Malaysia and Pakistan (Sharma *et al*., 2017). The prevalence of thalassaemia in Iraq has increased from 35.5/100,000 in 2010 to 37.1/100,000 in 2015 (Kadhim *et al*., 2017). Judging by red cell indices and HbA_2, beta thalassaemia trait was found to be 26% in Nigeria population (Kotila, 2013), while a prevalence of alpha thalassaemia among sickle cell disease patients was found to be 41% (Olatunya *et al*., 2018).

PATHOPHYSIOLOGY

Haemoglobin molecule is a hetero-tetramer that comprises of two alpha (α) chains and two non-alpha chains which could either be Beta (β), gamma (γ) or delta (d) chains. Alpha (α) and Beta (β) chains form the major adult haemoglobin (HbA) (Ali *et al*., 2019), while α and d chains form a minor fraction of adult haemoglobin (HbA_2) (Angastiniotis and Lobits, 2019). Finally, α and γ chains form the fetal haemoglobin (HbF) (Angastiniotis and Lobits, 2019). The gene that produces α globin is located on chromosome 16 while the gene that produces β, γ and d globin is located on chromosome 11 (Angastiniotis and Lobits, 2019). In normal physiological condition, there is a balance in the production of α, β, γ and d chains in order to ensure a reciprocal pairing into the normal tetramers (Angastiniotis and Lobits, 2019). Any imbalance as a result of ineffective production of one of the globin chains causes thalassaemia (Angastiniotis and Lobits, 2019). If α globin chains are not adequately produced, accumulation of β globin chains will result and this will cause Alpha thalassaemia (α thalassaemia) disease (Angastiniotis and Lobits, 2019). In the other hand, if β globin chains are inadequately produced, α globin chains will accumulate and this will result to Beta thalassaemia syndrome (Angastiniotis and Lobits, 2019).

Anaemia is a common occurrence among patients suffering from thalassaemia. The most common complications experienced by patients, especially those on regular transfusion as a result of severe type, are heart and liver damage, and destruction of the endocrine system as a result of iron overload leading to constant blood transfusion (Mishra and Tiwar, 2013). There is high risk of infection, which is as well detrimental to body organs. An abnormal body development may be observed in the bones of the skull and face which becomes thicker (Galanello and Origa, 2010). Splenomegaly and liver failure may occur due to circulatory overload (Galanello and Origa, 2010). Thalassaemia patients can also experience shortness of breath, cold hands and feet, pale skin, irritability, fatigue, dark urine etc due to anaemia

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(Shirzadfar and Mokhtari, 2018). The extent to which globin chain reduction takes place, is ascertained by the nature of the mutation at the beta globin gene located on chromosome 11 (Huang *et al*, 2020) The nature of mutation could be the reduced amount (β+) or absence (β0) of beta globin chains that precipitate in erythroid precursors in the bone marrow, causing their premature death which brings about ineffective erythropoiesis (Langhi Jr. *et al*., 2016).

Anaemia that is occasioned by thalassaemia stimulates the production of erythropoietin with resultant intensive, but ineffective expansion of the bone marrow (up to 25-30 times normal), which therefore leads to the typical bone deformities (Tha and Tha, 2014). Persistent and severe anaemia with high erythropoietic drive also brings about hepatosplenomegaly and extramedulary erythropoiesis Galanello and Origa, 2010). The presentation of the disease is dependent on the severity of the diseases (Lecturio Medical Online Library). For example, there is observable yellow discoloration of the skin in thalassaemia major state (Shirzadfar and Mokhtari, 2018). Also observed are weakness, abdominal distension, dark urine (as a result of haemolysis) and poor appetite (Thakur and Raw, 2018). Multi-transfused thalassaemia major patients always come down with severe endocrine complications majorly as a result of iron overload, anaemia and chronic liver disease that need early diagnosis, treatment and follow-up by specialist (Mishra and Tiwar, 2013).

CLASSIFICATION OF THALASSAEMIA

There are two major forms of Thalassaemia: Alpha (α) and Beta (β) thalassaemia (Abu-shaheen *et al*., 2020).

ALPHA THALASSAEMIA

This results from decrease in production of α_1 or α_2 globin chain leading to the accumulation of beta chains resulting in less stable chains. Alpha-globin gene is present in duplicate on chromosome 16 and the mutation of the gene leads to clinical disease known as alpha thalassaemia (Abu-shaheen *et al*., 2020). Molecular study on alpha thalassaemia indicates that the disease is majorly a result of removal of changeable pieces from one or two alpha genes. The severity of the disease is dependent on the number of genes involved. If a patient is lacking one of alpha gene release, α^{+}htα 1 results, but if two α genes are missing, α^{+} Gtα 1^{+} occurs. In this form, patients are asymptomatic or associated with intermediate thalassaemia with intermediate anaemia. Deletion of each of the 4 genes leads to Hydrops Fetalis disease that is associated with intermediate intra uterine foetal death (Tari *et al*., 2018). Over 95% of alpha thalassaemia is kind of elimination. The most rampant types whereby two genes are deleted, are the South East Asian Variety (--SEA), Mediterranean (--MED) and Philippine (--FIL) types respectively, while the most common types involving the removal of a single gene, are known as -α 3.7 and -α 4.2 (Tang *et al*., 2001). Deletions of 1 or 2 α-globin genes, also known as salient carrier state and α-thalassaemia trait respectively, do not commonly result to any symptom, but the loss of 3 genes causes mild to severe anaemia, which may be complicated by fever or different infections. However, the loss of all 4-α-globin genes occurring most frequently in some parts of Asia, may lead to fetal death (Kessler, 2014).

BETA THALASSAEMIA

Beta thalassaemia is that type of thalassaemia resulting from reduced quantity and quality of beta globin chain production. This causes low quantity of beta globin chains which in turn, brings about excess of alpha globin chains, thereby causing a more severe early apoptosis and resultant ineffective erythropoiesis in the bone marrow (Chuncharunee *et al*., 2019).

TYPES OF BETA-THALASSAEMIA

1. Thalassaemia minor (also known as Beta thalassaemia carrier, or Beta thalassaemia trait), is a lesser form in which one normal copy of the Beta globin gene is present. Examples include are B_{+}/B and BO/B types (Tha and Tha 2014).

2. Thalassaemia intermedia: A type with clinical manifestations and heterozygous genetic mutations that still make room for some beta chain production. Example: B_{+}/BO, B_{+}/B_{+} (Tha and Tha 2014).

3. Thalassaemia major (also known as Cooley's anaemia), is a severe clinical phenotype that results when patients are homozygous or compound heterozygous for more severe beta chain mutation. Example: severe B_{+}/B_{+} mutations, B_{+}/BO, BO/BO) (Abu-shaheen *et al*., 2020).

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Alpha thalassaemia is predominant among Nigerians/Africans due to Sickle Cell Anaemia (SCA) which is predominant in the region when compared with other regions of the world (Olatunya *et al*., 2018)

DIAGNOSIS OF THALASSAEMIA

Clinically, thalassaemia major is suspected in infants with the following: 1) severe type of microcytic anaemia, 2) mild jaundice and 3) hepatosplenomegaly (Galanello and Origa, 2010). Thalassaemia intermedia occur at a later age with mild and similar clinical findings as in the thalassaemia major (Galanello and Origa, 2010), while thalassaemia carriers are usually asymptomatic but can develop mild anaemia from time to time (Galanello and Origa, 2010).

Nevertheless, laboratory diagniosis of thalassaemia can be diagnosed using different laboratory investigation techniques such as the like thin blood smear, amniotic fluid investigation during antenatal (as part of prenatal testing), DNA analysis in genetic testing, complete blood count (CBC), iron studies and haemoglobinopathy (Ali *et al*, 2019). In other words, the methods for the diagnosis of thalassaemia include complete blood count and differential, haemoglobin estimation and molecular genetic analysis (Brancaleoni *et al*., 2016).

COMPLETE BLOOD COUNT AND EXAMINATION OF THE BLOOD FILM

Haematologically, thalassaemias are generally classified as hypochromic and microcytic anaemias (Clarke and Trefor, 2000), Galanello and Origa, 2010).

In thalassaemia major, full blood count shows Hb< 7gldl, mean call volume (MCV) >50<70 fl, and Mean Corpuscular Haemoglobin (MCH)>12<20pg (Origa, 2017). Anisocytosis and poikilocytosis, target cells and ovalocytes are characteristics. Occasionally, fragmented RBC, basophilic stippling, polychromasia, howell-Jolly bodies and circulating Nucleated Red Blood Cells (NRBCs) are observed (Origa, 2017).

In Thalassaemia Intermediate, full blood count shows Hb= 7-10 g/dl, MCV between 50 and 80fl and MCH between 16 and 24pg (Galanello and Origa, 2010).

In Thalassaemia Minor, there is reduced MCV and MCH, with increased HbA_2 level. Normal RBC count and target red cells are observed (Origa, 2017).

In alpha-thalassaemia trait, complete blood count (CBC) may show mild hypochromic anaemia and microcytic anaemia, with low MCH and MCV (Chand and snower, 2019) In other words, hypochromic microcytic red cells are observed (Harteveld and Higgs, 2010; Chand and Snower, 2019).

For alpha thalassaemia carrier, CBC shows either normal or mild reduction of MCV and MCH; though usually asymptomatic (Chand and Snower, 2019).

In HbH Disease, hypochromia, basophilic stippling, target cells and anisopoikilocytosis are observed (Chand and Snower, 2021). RBCs supravital stains reveal HbH inclusions occasionally accompanied by Hb Bart's in the peripheral blood (Harteveld and Higgs, 2010). Complete blood count shows reduced MCV and MCH, reticulocytosis (4-5%) and raised red blood cell count (Chand and Snower, 2019).

For HbBarts (hydropsfetalis) syndrome, microcytic hypochromic RBCs and severe anisopoikilocytosis are characteristics (Chand and Snower, 2021). CBC shows severe microcytic hypochromic anaemia and reticulocytosis (Chand and Snower, 2019). Molecular methods of diagnosing thalassaemia may reveal some results in rare mutation and recent DNA analysis for haemoglobinopathies are based on PCR methods that can detect the globin gene mutations (Tuli and Yenilmez, 2018).

ELECTROPHORESIS

Electrophoresis method using cellulose acetate electrophoresis, and DE-52 micro chromatography or high power lipid chromatography (HPLC), identifies the amount and type of Hb present. The Hb patterns in beta thalassaemia vary according to beta-thalassaemia type (Galanello and Origa, 2010).

Normally, electrophoresis has been the method of choice for identification and qualification of variant Haemoglobins. Rapid electrophoretic methods have been developed, making room for separation at Ph 8.4 and 6.2 on agarose gels (Clark and Trefor, 2000). These give a

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clear background and allow quantification of Hb by densitometric scanning. The bands are seen by staining with amino black and acid violet. At Ph 8.4, Electrophoretic migration of haemoglobin C, E, A_2 and O are similar. Migration of S, D and G are also similar, but at acid PH, haemoglobin C is separated from E and O, then haemoglobin S is separated from D and G. This is one of the limitations of the method. Other limitations include: 1) slow and laborious and 2) inaccurate quantification of low concentrations of Hb variants like HA_2 or in the detection of fast Hb varieties like Hb H and Hb barts, which are indicative of thalassaemia (Clarke and Trefor, 2000).

OTHER DIAGNOSTIC METHODS

Other methods for diagnosis of thalassaemia include Iso Electric Focusing (IEF), Capilary IEF, High Performance Liquid Chromatography (HPLC), DNA analysis. Interestingly, the gold standard for diagnosing thalassaemia is by DNA analysis based on the type of mutation that occur, while thalassaemia carriers are identified by an increase in HbA_2 levels (Siswandari *et al*., 2019).

CHALLENGES AND LIMITATIONS

Both alpha and beta thalassaemias are more prevalent in tropical and subtropical regions of the word especially where malaria is endemic (Yolanda, 2021). There is coexistence of alpha thalassaemia among young SCA Nigerian patients (Olatunya *et al*., 2018). Giving the fact that there is high incidence of malaria and SCA in Nigeria, and indeed in Africa as a whole (Olatunya *et al*., 2018), the prevalence of thalassaemia may be high. More so, diagnosis may be missed since it is not a routine test for patients and considered not a priority in genetic counselling in Nigeria. Notwithstanding, hypochromia and microcytosis associated with the disease may be misdiagnosed as iron deficiency anaemia (Kotila, 2013). Definitive diagnosis is also expensive and as such, the actual information on thalassaemia in Nigeria may be lacking.

PREVENTION OF THALASSAEMIA

The reason for prenatal diagnosis is to detect and counsel asymptomatic people whose children have the disease (Lee *et al*., 2019). Prevention of thalassaemia is firstly by prenatal diagnosis and this can be done by traditional conventional methods like amniocentesis; though the methods have the risk of foetal miscarriage (Tuli and Yenilmez 2018). Various techniques for the identification of foetal haemoglobinopathies are mass spectrometry, next-generation sequencing and genotyping assay, which are still challenging. It is therefore important that more studies are undertaken to develop and validate them for efficient, precise and reliable non-invasive prenatal diagnosis of thalassaemia and haemoglobinopathies (Lee *et al*., 2019).

TREATMENT OF THALASSAEMIA

Patients with thalassaemia slowly accumulate high levels of iron with many biochemical complications (Shirzadfar and Mokhtari, 2018). This could be as a result of massive blood transfusion which is currently one of the treatment options for severe thalassaemia, thereby causing iron overload (Sharma *et al*., 2017). Bone marrow transplantation and gene therapy is the only treatment that brings about permanent cure (Ali *et al*., 2019 and Sharma *et al*., 2017), but this is highly expensive and compatible donors are not easy to come by (Thakur and Raw, 2018). Blood transfusion is considered in more severe forms as deferoxamin deferiprone or sirox are used to prevent the breakdown of haemoglobin (Shirzadfar and Mokhtari, 2018). While Hb Bart's syndrome is universally fatal and death occurs in neonatal period, most individuals with thalassaemia carriers are clinically well and live without treatment (Chand and Snower, 2019).

The decision to start transfusion in patients with confirmed diagnosis of thalassaemia should be dependent on the presence of severe anaemia with Hb less than 7 g/dl for more than two weeks excluding other causes like infections (Langhi Jr *et al*., 2016 and Galanello and Origa, 2010). However, patients whose Hb is more than 7g/dl but have other issues like retarded growth, facial changes and splenomegaly should also be considered for transfusion (Langhi Jr *et al*., 2016). The decision for transfusion should begin as soon as necessary (Galanelo and Origa 2010). The most widely accepted, aims at a pre-transfusion Hb level of 9-10 g/dl and post-transfusion level of 13-14 g/dl (Galanello and Origa, 2010). This is to prevent retarded growth, organ damage and bone deformities as well as allowing normal activity and quality of life (Thalassaemia International Federation, 2018).

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The frequency of transfusion is usually two to five weeks (Langhi Jr *et al*., 2016). Iron overload is the most relevant complication linked with the red cell transfusions Galanello and Origa, 2010). Iron chelators are used to remove excess iron (Galanello and Origa, 2010). Deferoxamin (DFO) is an iron chelator used to decrease morbidity (Galanello and Origa, 2010). Another iron chelator of importance is deferiprone (DFP) (Shirzadfar and Mokhtari, 2018 and Sharma *et al*., 2017), which has shown efficacy in reducing myocardial siderosis in thalassaemia major (Piga *et al*., 2003). Other iron chelators are Deferasirox (DFX), (S) 3-(OH)-desazadesferrithiocin-polyether, magnesium salt (Galanello and Origa, 2010). However, treatments like splenectomy, bone marrow and cord blood transplantation can be applied when necessary and possible (Galanello and Origa, 2010).

Other treatments used in sickle cell disease are effective in thalassaemia patients. Such treatments are foetal haemoglobin (HbF) reactivation by 5 azacytidine, butyrate and Hydroxyurea (Kotila, 2013).

CONCLUSION

Thalassaemia is a significant public health burden in affected regions. Therefore, prenatal screening and genetic counselling are important in preventing the most severe forms. Two major forms of thalassaemia have been identified –the alpha and beta, and both are subdivided according to the level of mutation on both alpha and beta globin chains resulting to minor or major forms of thalassaemia. Presentations may be mild, moderate or severe, as the case may be. Diagnosis is through complete blood count, examination of the blood film as well as electrophoresis. Current standard diagnosis as well as more novel molecular techniques that have recently become available is a major breakthrough e.g. DNA analysis. Current treatment like haematopoietic stem cell transplantation and other maintenance treatment may lead to long survival and good quality of life. Bone marrow transplantation is the only treatment that brings about cure but this is highly expensive so much as only a few patients are able to find compatible donors.

RECOMMENDATIONS

We recommend the following:

1. Considering the high incidence of malaria and SCA in Nigeria, every patient with microcytic hypochromic anaemia should among others, be equally screened for thalassaemia in order not to miss it out.
2. Automated haematology analysis should be used for complete blood count so as to ascertain the red blood cell indices which are very important in the screening of thalassaemia.
3. Laboratory staff should be trained on the molecular diagnosis of thalassaemia which is the gold standard for definitive diagnosis.
4. Premarital counselling should include thalassaemia so as to prevent carriers from producing thalassaemia children.
5. Government of Nigeria should consider a program to address the issue of thalassaemia.

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