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Prolific Drug Discovery Human B-Hemoglobiopathies B Thalassemia - Sickle Cell Anemia-Anemic Condition

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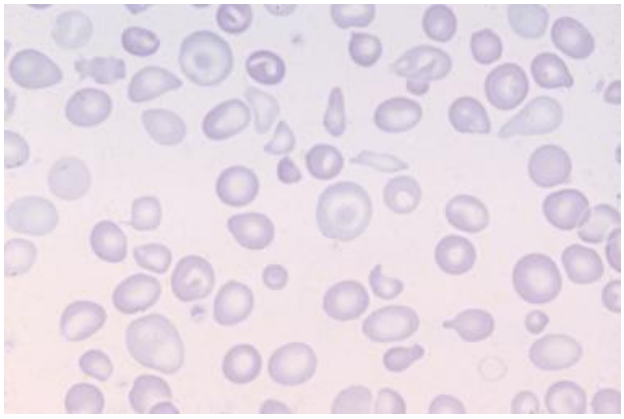
1. Introduction

1.1 Types of Thalassemia

There are three types of Thalassemia, such as Alpha (α) Thalassemia, Beta (β) Thalassemia and Delta (δ) Thalassemia.

The Thalassemia are the commonest monogenic disorders in the world, and globally it is estimated that there are 270 million carriers, of which 80 million are carriers of β-Thalassemia. B-Thalassemia is widespread in the Mediterranean, Southeast Asian, African, and Middle East populations. The mean prevalence of this disease in India is 3.3%. It has become much more common recently in northern and central Europe, including Germany, due to immigration . The Thalassemia refer to a diverse group of hemoglobin disorders characterized by a reduced synthesis of one or more of the globin chains (α, β, γ, δβ, γδβ, δ, and εγδβ). B-thalassemia occurs when there is a deficiency of β-globin, which is typically caused by a direct down-regulation in the synthesis of structurally normal b chains. However, a thalassemia phenotype can also arise from structural b chain variants if they are synthesized at a reduced rate. The most severe form of Thalassemia is characterized by the complete absence of HbA ($\alpha_2\beta_2$) which results from the inheritance of two homozygous β-thalassemia alleles. This normally presents as a life-threatening anemia requiring blood transfusions from infancy. Inheritance of single β-thalassemia alleles is presented by a clinically asymptomatic condition, but may show a mild anemia

Genetic disorders caused by mutations in the β-globin gene are widely known as the human β-hemoglobinopathies, in which β-thalassemia and sickle cell disease (SCD) are the most prevalent ones, particularly in the Mediterranean, Africa, and Southeast Asia, leading to great mortality and morbidity [1–4]. The high occurrence of the β-thalassemia and SCD mutations is due to the reason that both cause mild severity of malarial infection in the heterozygous state . However, in the homozygous state, these mutations shorten the lives of affected ones.

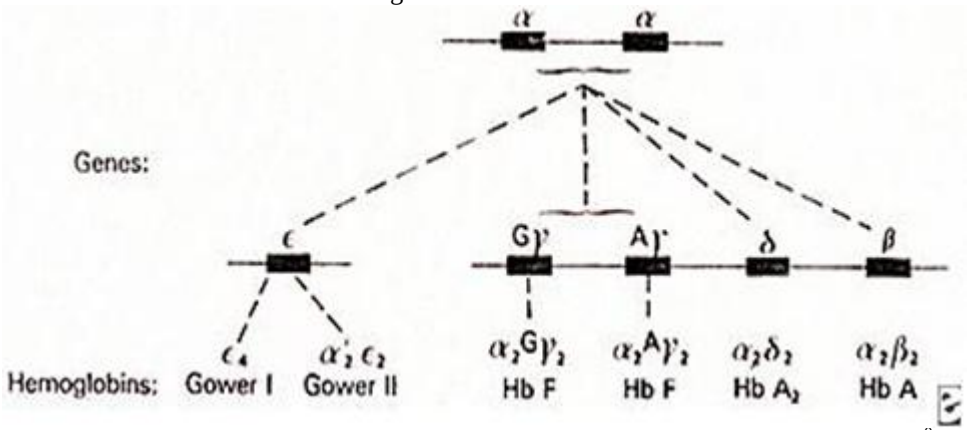


B Thallasemia

B-thalassemia is caused by the inherited mutations in the β-globin gene complex, resulting a total absence or severe decrease in the production of β-globin chains. The lack of β-globin chain production leads to the accumulations and precipitations of free intracellular α-globin chains, which may consequently result in premature hemolysis of red blood cells and apoptosis of erythroid precursor. Ineffective erythropoiesis has also been known to be related to inefficient iron utilization. Therefore, the combining effects of ineffective erythropoiesis, hemolysis, and hypersplenism are the main culprit of severe anemia in β-Thalassemia patients is an inheritable autosomal recessive genetic blood disorder. It is characterized by the abnormal appearance of the red blood cells which are rigid and sickle. SCD is attributed to a point mutation at the coding sequence of the β-globin gene which causes the substitution of glutamate by valine in the glutamic acid at the sixth position of β-globin protein, and thus forming sickle hemoglobin (HbS,) when incorporating into hemoglobin tetramer]. HbS will polymerize inside the red blood cells under hypoxic condition, resulting in the alternation of the shape of red blood cells as well as their function.

Apart from gene therapy, fetal hemoglobin reactivation by chemical agents appears promising enough to develop into effective interventions to cure human β-hemoglobinopathies. Previous studies have revealed that homozygous β-thalassemia patients will not suffer severe anemia until fetal γ-globin genes are silenced and that patients carrying hereditary persistence of fetal hemoglobin (HPFH), meaning fetal hemoglobin (HbF) is abnormally persisted at high level in adults, will only suffer mild anemia More evidences also supported that HPFH can improve the severity of both β-thalassemia and SCD [1]. Therefore, it have been suggested

that increasing the synthesis of fetal hemoglobin (HbF) by reactivating fetal γ-globin gene can be a potential therapy in patients suffering β-thalassemia or SCD. It is expected that the pharmacological induction of HbF can correct the globin chain imbalance in β-thalassemia patients, while inhibit HbS polymerization in SCD patients [28–32].



Chemotherapeutic agents, such as 5-azacytidine, hydroxyurea, myleran, and butyrate, had long been used for β-thalassemia treatment by stimulating HbF synthesis; yet, cytotoxicity, growth-inhibitory effect, fear of long-term carcinogenesis, and only modest HbF-inducing activity have limited the clinical usage of these agents in β-thalassemia and SCD treatment. Also, through understanding the pathology of β-thalassemia, it is revealed that most of the identified HbF-inducing agents have limitation on treating β-thalassemia. It is because the rapid cellular apoptosis of erythroid progenitors in β-thalassemia causes a significant obstacle that over stimulating the cell stress signaling pathway by the HbF inducer may possibly lead to irreversible cellular apoptosis before γ-globin gene expression and HbF synthesis can be stimulated. With the advancement of biotechnologies, increasing number of studies will be done to explore and optimize new interventions and nature remedies to reactivate HbF synthesis for β-thalassemia patients. In the future, it is expected that increasing number of HbF inducing agents could be found from natural remedies and folk medicines all over the world. In this context, further studies are required with the aim of exploring more natural herbal medicines as well as studying the efficacy and safety of from the laboratory to clinical use for the individuals with β-hemoglobinopathies.

1.2 Genetic Background

Hemoglobin comprises four globin chains: fetal hemoglobin (Hb F) has two α and two gamma chains (α2γ2) and adult hemoglobin (Hb A) has two α and two β chains (α2β2). Genes in the α-globin and β-globin gene clusters (on chromosomes 16 and 11) control globin-chain production. Due to spontaneous mutation, haemoglobin gene variants are present at low prevalence (carriers 1–1.5/1000) in all sizeable populations. They fall into two broad groups – structural variants that change the amino acid sequence and produce unusual haemoglobin, thalassaemias that lower or abolish production of globin chains Most haemoglobin gene variants are rare and many are harmless, but some are common because carriers are less likely than others to die from falciparum malaria. The most common such variant, α plus (α^+) thalassaemia, is usually harmless. However, people who inherit combinations of haemoglobins S, C, E, D Punjab, β thalassaemia, or α zero (α^0) thalassaemias may have a serious haemoglobin disorder. In populations in which malaria is (or was) endemic, 3 to 40% of individuals carry one of these significant variants, and the prevalence of haemoglobin disorders ranges from 0.3 to 25 per 1000 live births.

1.3 Global Burden of Haemoglobin Disorder

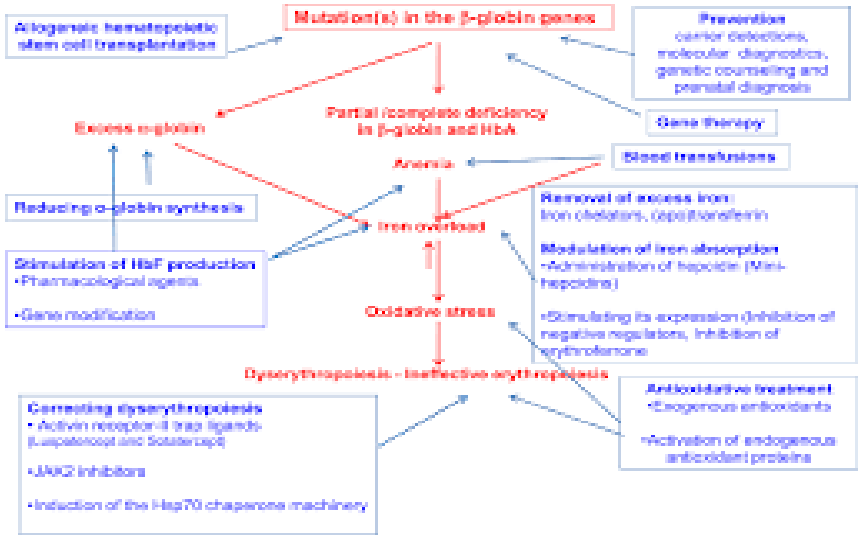
The yardstick of under-5 mortality can be used to assess the broad effect of haemoglobin disorders on health, because most affected children die in early childhood and most survivors have chronic disease. Table 1 shows that they cause the equivalent of at least 3.4% of deaths in children aged less than 5 years. However, this still underestimates their burden because inherited disorders affect families. Worldwide, over 1% of couples are at risk for hemoglobin disorders, most have at least one affected child, and most affected children die in early childhood. Although the west African death rate in children aged under 5 years is 18.4%, the rate is 16.5% for children born to couples not at risk for sickle-cell disorders compared with 40% for children born to couples who are at risk. Clearly, methods to assess the health burden of inherited disorders must include a family perspective.

2. Objective

(Drug Discovery Focus)

1) To increase the hemoglobin levels
2) Increase the interval between blood transfusions and decrease the amount of total blood transfused in patients with thalassaemias major.
3) Erythroid differentiation of K562 cells, HbF
4) DNA-binding activity – Erythroid progenitors & *y* – globulin, mRNA accumulation of human leukemia K562 Cells.
5) Stimulatory effect on the HbF production in primary Erythroid Prohenitor stem cells (EPSCs)
6) Immunity
7) Erythropoiesis, cell proliferation, and transcription
8) DNA-binding activity – Erythroid progenitors & *y* – globulin mRNA accumulation of human leukemia K562 Cells.

9) Helps to reduce frequency and risk of Blood Transfusions.
10) To reduce economic burden.
11) Natural chemical entities may decrease jeopardy of side/adverse effects, Comparatively with the conventional treatment.
12) Helps to elevate hemoglobin level.



The need for prevention of thalassaemias is obvious due to high frequency of the condition, the great expense and difficulties in providing optimal treatment for patients, and the innumerable fatalities from untreated β-thalassaemia. Prevention would not only be a good public health practice, as envisioned in Alma Ata declaration, but it would also be cost-effective, as the ratio of the cost of treatment to prevention is 4:1, as shown in a study from Israel. It would help tremendously in reducing the burden of the disease for patients, families and the health services. The strongest argument for prevention is that it would ensure the best possible care for the affected, by curbing the increase in their number.

It is now inevitable to discover safer, effective and cost effective therapy: drug/supplement to combat the disease, thalassaemias, sickle cell anemia and anemic conditions.

Tropical forest plants have served as a source of medicines for people of the tropics for millennia. We are well aware of the number of modern therapeutic agents that have been derived from tropical forest species. It is a fact of history that around 120 modern pharmaceutical products have been derived from plants and some 75% of these were discovered by examining the use of these plants in traditional medicine.

Until the early '70' s there was a strong interest in looking at plants as sources of new pharmaceutical agents in the International pharmaceutical agents in the International Pharmaceutical industry. By Contrast, 1970's and the early 1980's breakthrough in molecular biology and genetic engineering has created new promises in pharmaceuticals without the need for nature's chemical diversity.

It is well-known among natural product chemist and phytochemists that plant species contain a bewildering diversity of secondary metabolites. Individual plant species often contain over 1000 unique chemical entities or the enzymatic machinery needed to produce compounds upon the proper stimulus. One of the most compelling explanations for this vast array of chemical diversity, which resides within the biological diversity of tropical species, is found in the relatively new science of chemical ecology.

Isolating, identifying and developing a novel therapeutic agent from a tropical forest species involve its own special set of challenges. Most of the highly sensitive and selective receptor and enzyme based assays do not respond well to mixture of compounds that are found in plant extracts. The usual manifestations are false positive and / or lack of reproducibility. The former problems arises from ubiquitous classes of compounds such as tennis that can create false positives or mask other bioactivity by protein binding, for example, saponins acting as a detergent. The lack of constant and reproducible solubility of extracts is another potential interference responsible for the latter. It is clear that unless extracts of plants are carefully created and handled, active can be lost or missed.

3. Conclusion

A comprehensive and multidisciplinary therapy has to be focused. I have considered formulating selected, safe and effective chemical entities to embark towards prolific drug/supplement discovery based on plants.
The following phytochemical have high potential to combat disease:

1) Chlorophyll - (Wheat Grass) –Chlorophyll A
2) Bergatene - (Aegal marmelos)
3) Cissus quadrangul - Extract
4) Cucurbitacin - (Fructus trichosanthis)
5) C.Phycocyanin - (Algae – Spirulina)
6) Termanalia catappa - Extract with active (Leaves)
7) Curcumin 95 % - (Curcuma longa)
8) Ursolic acid 2 % or more- Ocimum sanctum

Three or more above ingredients/ molecules may be used for the aforesaid treatment.

Treatment strategies can be based on the following factors: an understanding pathophysiology of the disease; perspective of HbF to alter its manifestation; and that the developmental changes in γ-globin gene can be reversed by controlling cellular and molecular regulatory mechanism [21]. Different classes of HbF inducers for clinical use

These chemical entities will be formulated with its specific weight and volume.

The route of administration will be oral with capsule, soft gelatin capsule, caplet, tablet, syrup sachet and/or granules. It is further also postulated that the supplement/drug may be made as "Sustained Release" for more potent and sustainable efficacy. The strength of dosage will be varied for multiple purposes.

Comments: This prolific drug discovery project is ensured positively for the financial aid by family and friends. The pilot case study on 17 years old girl has encouraged embarking further for conclusive outcome as she had been removed beta Thallasemia with sustainable result.

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