Journal of Applied Sciences Research, 7(3): 357-368, 2011
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Impaired Arginine-Nitric Oxide Pathway and Pulmonary Hypertension in Thalassemia and Sickle Cell Hemoglobinopathies-(*Review article*)

[1]Mohamed Mostafa El Missiry, [2]Ali Belkasim Makhlouf

[1]Department of Internal Medicine, National Research Center, Cairo, Egypt
[2]Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

ABSTRACT

Thalassemia and sickle cell anemia are beta globin defect hemolytic anemias characterized by pulmonary hypertension (PHT) which cause, or, aggravate serious cardiovascular complications in these diseases. Combined pathological mechanisms resulting from hemolysis, oxidative stress and coagulopathies, lead to portal hypertension in these diseases. On consequence of hemolysis, released hemoglobin from destructed erythrocytes scavenges nitric oxide (NO) synthesized by endothelium. Moreover, a deficiency of arginine, which is a precursor in NO formation through the nitric oxide senthase (NOS), also occurs due to the over-release of arginase enzyme from damaged erythrocytes. Oxidative stress condition and its reactive oxygen species (ROS) contributes to aggravation of NO decrease by additional scavenging of NO as well as accumulation of asymmetric dimethyl arginine (ADMA) which is a competitive inhibitor of nitric oxide senthese (NOS). Moreover, NO synthase itself may produce superoxide in lieu of NO when arginine concentration is low. Coagulopathies acts as another risk factor for portal hypertension as well. In consequence of all the previous, establishing ways to overcome arginine-NO pathway disturbances and the resulting pulmonary hypertension in thalassemia and sickle cell anemia is a appear to be fundamental challenge in dealing with these diseases. Administrations of L- arginine, L-citrullin and Nebivolol are suggested as solutions that are needed to be further studied. Complementary antioxiants could be very beneficial if it is also used in parallel.

Key words: Sickle cell- Thalassemia- Pulmonary hypertension- Arginine- Nitric oxide.

Introduction

Secondary pulmonary hypertension (PHT) is emerging as a significant cause of morbidity and mortality in patients with hemolytic anemia as it may cause or aggravate serious cardiovascular complications in such diseases. Thalassemia and sickle cell anemia are beta globin defect hemolytic anemias characterized by hemolysis, oxidative stress conditions and hypercoagulability state that lead to pulmonary hypertension with all its mortality and morbidity risks. The thalassemia syndromes have many biologic and epidemiologic factors in common with sickle cell disease (SCD), including chronic hypoxia, long-term effect of splenectomy, red cell membrane pathology coagulation abnormalities platelet activation, oxidative stress, iron overload and chronic hemolysis (Morris and Vichinsky, 2010). As a result of hemolysis process, hemoglobin molecules are released from these destructed erythrocytes. This released hemoglobin scavenges nitric oxide (NO) synthesized by the endothelium, and consequently a state of vaculopathy and portal hypertension as a result of decrease NO occurs. Arginine is an amino acid which is a precursor in the formation of nitric oxide by the enzyme nitric oxide synthase (NOS) (Orea-Tejeda *et al*., 2010). A deficiency of arginine also occurs due to increase in activity of arginase enzyme as a result of its over-release from damaged erythrocytes. The free iron, another erythrocyte component, leads to a state of oxidative stress in these patients with the formation of the harmful reactive oxygen species (ROS) such as superoxide and peroxynitrite. Oxidative stress condition also contributes to aggravation of NO decrease by additional scavenging of NO and its consumption in the formation of harmful peroxynitrite. Reactive oxygen species (ROS) result also in the accumulation of asymmetric dimethyl

Corresponding Author: Mohamed Mostafa Mohamed Ahmed El Missiry, Department of Internal Medicine, National Research Centre, El Buhooth Street, Dokki, Cairo, Egypt.
Tel: 0020111214689,
E-mail: drmissiry@yahoo.com

arginine (ADMA) which is a competitive inhibitor of NOS. Moreover, NO synthase itself may produce superoxide in lieu of NO when arginine concentration is low (Morris *et al*., 2003; Morris, 2006; King *et al*., 2008). Coagulopathies resulted from chronic depletion of nitric oxide and arginine in hemolytic anemias as well as splenectomy acts as another risk factor for portal hypertension as well (Morris, 2008; Gladwin and Vichinsky, 2008).

L-Arginine

L-arginine is a semi-essential basic amino acid formed from citrulline and ornithine. It plays multiple roles in metabolism by serving as a substrate for protein synthesis, an intermediate in the urea cycle, and a precursor for the synthesis of various important metabolic molecules. Most importantly, it is a precursor in the formation of nitric oxide by the enzyme nitric oxide synthase. Another example, It is converted by arginase to L-ornithine, a precursor of polyamines and urea, which is important in the urea cycle. It also directly increases the formation of NO and indirectly stimulates the liberation of growth hormone. Effects are achieved with oral doses ranging from 5.6 to 12.6 g/day (maximum 30 g) (Tapiero *et al*., 2002; Mateo *et al*., 2007; Orea-Tejeda *et al*., 2010). Thus, dietary intake of L-arginine may be critically important in the context of how the human body responds to inflammation and oxidative stress. Recent studies suggest that supplemental L-arginine may be helpful in preventing harmful oxidation and reversing endothelial dysfunction. The most common dietary sources of L-arginine are meat, poultry and fish, dairy products, and nuts (King *et al*., 2008).

Arginase

Arginase is an essential enzyme in the urea cycle, responsible for the conversion of arginine to ornithine and urea. It can be induced in many cell types by a variety of cytokines and inflammatory stimuli (Morris, 2006). Two forms of arginase have been identified: type 1, a cytosolic enzyme highly expressed in the liver, and type 2, a mitochondrial enzyme found predominantly in the kidney, prostate, testis, and small intestine. Arginase-1 is also present in human red blood cells. Plasma arginase activity is elevated in SCD as a consequence of inflammation, liver dysfunction and, most significantly, by the release of erythrocyte arginase during intravascular hemolysis, which has been demonstrated by the strong correlation between plasma arginase levels and cell-free hemoglobin levels and other markers of increased hemolytic rate. In addition, arginase activity is higher in the erythrocytes of patients with thalassemia and SCD compared to normal controls, and strongly correlates to plasma arginase activity. Up-regulated expression of arginase-1 also results in increased proliferation rates of vascular smooth muscle and endothelial cells and in this capacity, may further contribute to vasculopathy in addition to its unique role during hemolysis (Morris, 2008).

L-Arginine in NO Synthesis: (L-arginine, Nitric oxide synthase and Nitric oxide)

As previously mentioned, The main importance of arginine (Arg) is attributed to its role as a precursor for the synthesis of nitric oxide (NO), which is a free radical molecule that is normally produced by the endothelium (Gladwin and Vichinsky 2008) in all mammalian cells from L-Arg by NO synthase (NOS) (Tapiero *et al*., 2002).

Synthesis of NO requires L-arginine and NADPH, and results in the formation of citrulline (Fig 1). NO synthesis requires not only these substrates but also four other coenzymes/cofactors (Protoporphyrin IX haem, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), tetrahydrobiopterin (BH_4)), as well as the presence of calmodulin. Subsequent work demonstrated that molecular oxygen is also a substrate for this reaction, being incorporated into both NO and citrulline (Knowles and Moncada, 1994).

Importance of Nitric Oxide (NO)

NO is one of the most potent vasodilators known, and is essential to vascular homeostasis. It plays an important role in the maintenance of vasomotor tone, limits platelet aggregation and ischemia-reperfusion injury, modulates endothelial proliferation, It additionally has anti-inflammatory properties and mediates immune response (Morris, 2006). NO also plays a role as a neurotransmitter, and as signaling molecule (Tapiero *et al*., 2002).

NO which appears to be a major form of the endothelium-derived relaxing factor (EDRF). NO and EDRF share similar chemical and pharmacological properties and are derived from the oxidation of a terminal guanidine group of L-Arg. Various mechanisms have been implicated in the defect in vascular relaxation. These

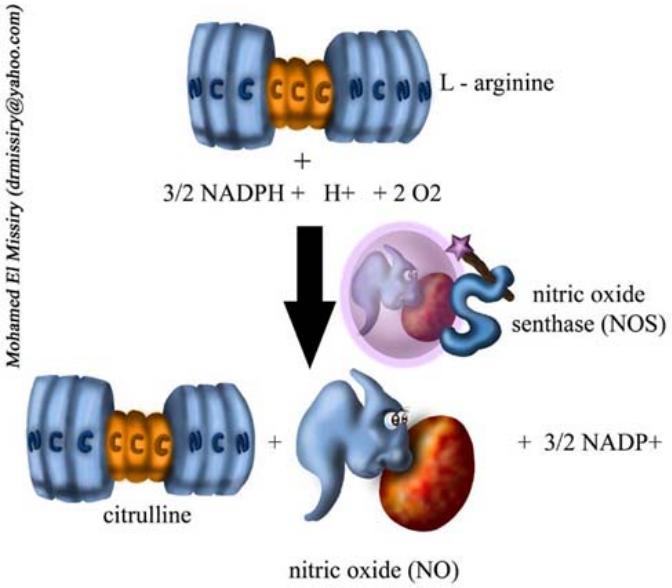

Fig. 1: Nitric Oxide synthesis.

include, L-Arg depletion, increased diffusional barrier for NO, altered levels of reactive oxygen, inactivation of NO by superoxide anions (O2-). The independent reactions of O2-, NO and their reaction yielding peroxynitrite (ONOO-) are critical in the initiation and maintenance of the atherosclerotic state and contribute to the defect in vasorelaxation (Tapiero *et al*., 2002).

Nitric oxide Synthases (NOS)

The enzymes responsible for the synthesis of NO from L-arginine in mammalian tissues are known as NO synthases (NOS) (they are not synthetases as the reaction does not utilize ATP) (Knowles and Moncada,1994). There are three types of NOS Isozymes which are neuronal type (nNOS or NOS-1), cytokine-inducible type (iNOS or NOS-2) and endothelial type (eNOS or NOS-3). iNOS and nNOS are soluble and found predominantly in the cytosol, while eNOS is membrane associated (Knowles and Moncada, 1994).

Hemoglobinopathies (Disorders of Hemoglobin)

β- Thalassemia:

This is genetic disorder disease that results from a reduced rate of synthesis of beta globin chains (Hoffbrand *et al*., 2006). The silent carrier state, ß-thalassemia trait are generally asymptomatic, whilst the intermedia and major states are the ones which we concern, provided that, the former state is more sever than the latter. A case in a point, thalassemia major patients needs regular transfusion starting from the first year of life, while intermedia patients need much less frequent transfusions (Camaschella and Cappellini, 1995; Olivieri, 1999).

There are two main lines in pathology of Beta thalassemia, namely anemia and iron overload. Both are consequences for the lack of production of adequate beta hemoglobin, together with accompanied overproduction of un-conjugated alpha hemoglobin. In the light of latter causes, several pathological disorders are resulted, including, Ineffective erythropoiesis, structural damage to erythropoetic cell membranes at both bone marrow and peripheral blood levels. Damage at peripheral level is called hemolysis of RBCs, and occurrence of microcytic hypochromic anemia (Eleftheriou, 2007; Lichtman *et al*., 2007).

Complications and undesirable sequels of beta-thalassemia are tremendously variable, including, hepatomegaly, splenomegaly, heart failure, pulmonary hypertension, bone disorders, coagulation disorders, infections and immunological abnormalities (Tripatara *et al*., 2007; Cappellini *et al*, 2008; Erdem *et al*., 2009).

Sickle Cell:

Sickle hemoglobin is a mutant hemoglobin in which valine has been substituted for the glutamic acid normally at the sixth amino acid of the β-globin chain. This hemoglobin becomes polymerized and becomes

poorly soluble when the oxygen tension is lowered and red cells that contain this hemoglobin become distorted and rigid. Sickle cell disease occurs when an individual is homozygous for the sickle cell mutation or is a compound heterozygote for sickle hemoglobin and β-thalassemia, hemoglobin C, and some less common β –globin mutations. Diagnosis depends upon demonstrating the presence of the abnormal hemoglobin(s) in the red cells. The disease is characterized by hemolytic anemia and by three types of crises: painful (vasoocclusive), sequestration, and aplastic. Complications include splenic infarction and autosplenectomy, stroke, bone infarcts and aseptic necrosis of the femoral head, leg ulcers, priapism, pulmonary hypertension, and renal failure. The severity of clinical manifestations varies greatly from patient to patient, and the aggressiveness of treatment needs to be modified accordingly (Lichtamn *et al*., 2007).

Pulmonary Hypertension (PHT) and Hemolytic Anemias

Secondary PHT is emerging as a significant cause of mortality and morbidity in patients with hemolytic anemia, for example, sickle cell disease, thalassemia syndromes, paroxysmal nocturnal hemoglobinuria, red cell membrane disorders, and alloimmune hemolytic anemia (Morris, 2010). PHT is defined as a mean pulmonary artery pressure of≥25mmHg at rest or≥30mmHg during exercise, and can result from a wide range of conditions. It is a vascular disorder of the lung in which the pulmonary artery pressure rises above normal levels, compromises oxygenation, causes right sided cardiac dysfunction, and can ultimately become life threatening. Early stages of PH in chronic hemolytic disorders may be asymptomatic or associated with mild symptoms. Classic symptoms of PHT like exertional dypsnea overlap with those of chronic anemia, often delaying clinical suspicion until late in the course of disease. In addition, the multiorgan complications of hemolytic anemia may limit exercise tolerance independent of elevations in pulmonary vascular resistance. Asthma may also be a cofounding complication in hemolytic anemia manifesting overlapping symptoms with pulmonary hypertension that requires screening. Hypoxemia, which could also be the result of restrictive lung disease, may be an early sign of pulmonary hypertension (Morris and Vichinsky, 2010).

Thalassemia and PHT:

PHT is increasingly recognized in thalassemia and can contribute to heart failure and death. Studies in both thalssemia intermedia (TI) and thalassemia major (TM) demonstrate that adults frequently have undetected PH, with a prevalence of 60–75% reported. Although the high prevalence of PH in nontransfused TI patients has now been well documented, mild to moderate PH was diagnosed in over 50% of patients with β -thalassemia despite transfusion (Morris and Vichinsky, 2010).

In terms of pathophysiology, it seems that pulmonary hypertension in β-thalassemia results from a rather complex combination of mechanisms, which lead to the increase of both cardiac output and pulmonary vascular resistance. Cardiac involvement represents the primary cause of mortality in both thalassemia major (TM) and thalassemia intermedia (TI). In this context, pulmonary hypertension is part of the cardiopulmonary complications of the disease (Aessopos, 2005). The etiology of PH in thalassemia is multifactorial. Chronic tissue hypoxia and chronic hemolysis are believed to hold the central pathogenetic role, while individual mechanisms involved include the prolonged anemic state, Advancing age, Hypoxemia, the increased percentage of hemoglobin F, the hepatic abnormalities, splenectomy, the presence of a hypercoagulabilable state, red blood cell membrane alterations, the thalassemia related elastic tissue defects, the coexistent endothelial dysfunction, oxidative stress, iron overload, chronic inflammation, low nitric oxide bioavailability, arginine dysregulation and arginase excess.(Aessopos *et al*., 2007; Cappellini *et al*., 2008; Morris and Vichinsky 2010).

Sickle cell and PHT:

Pulmonary hypertension (PHT) occurs in approximately 30% of adult sickle cell patients and is associated with a high risk of early death. Hemolysis driven reductions in nitric oxide (NO) bioavailability resulting from NO scavenging by cell free hemoglobin and increased arginase activity are of importance in the pathophysiology of SCD related PHT. (Landburg *et al*., 2008). The pathogenesis of PHT in SCD is probably due to a variety of factors. Firstly, it results from scavenging of nitric oxide by cell-free plasma hemoglobin. In sickle cell disease, the hemoglobin and heme scavenging systems are saturated and overwhelmed, even in the steady state (Gladwin and Vichinsky 2008). Secondly, it is due to elevated arginase activity as a consequensce of it's release following hemolysis. Arginase converts L-arginine to ornithine, and the resultant is decrease in the arginine/ornithine ratio which is found in PHT of SCD. Thirdly, although various studies have found no association between PHT and a history of acute chest syndrome, chronic lung injury resulting from repeated episodes of acute chest syndrome may lead to the development of PHT. This is due to chronic

fibrotic pulmonary parenchymal damage, altered vascular tone, vascular proliferation, hypoxia and consequent pulmonary vasculopathy. Finally, pulmonary thromboembolism and progressive endothelial damage with concentric pulmonary vascular intimal hyperplasia and in situ thrombosis may also contribute to the pathogenesis of PHT in SCD (Ataga *et al*., 2008).

Hemolysis

a) Hemoglobin and Nitric oxide Relation:

One of the most intriguing areas of research in erythrocyte physiology is the interaction of hemoglobin with nitric oxide (NO) (Allen and Piantadosi, 2006).

The half-life of nitric oxide in the blood is extremely short because of its rapid reaction with hemoglobin to form methemoglobin and nitrate. Under normal conditions, it has been found that there are two mechanisms that effectively prevent reaction and scavenging of NO produced by the endothelium. The first is the encapsulation of hemoglobin within the erythrocyte dramatically slows the consumption of NO external to the cell (Morris 2008, Robinson *et al*., 2005). The other mechanism is the existence of an erythrocyte-free zone adjacent to the endothelium in smaller blood vessels that spatially separates the red cell from the endothelium (called unstirred layer). These two causes reduce the rate at which nitric oxide reacts with intracellular hemoglobin by two to three orders of magnitude (Fig.2) (Robinson *et al*., 2005; Gladwin and Vichinsky 2008).

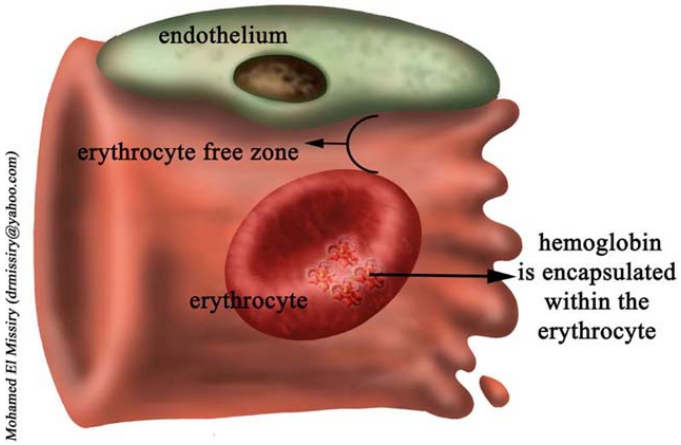

Fig. 2: The 2 Factors That Prevent NO Produced by Endothelium from Being Scavenged.

b) Hemolysis and the Arginine–Nitric Oxide Pathway:

The process of hemolysis initiates a global attack on the arginine-NO pathway. During hemolysis, hemoglobin is decompartmentalized and released into plasma, where it rapidly reacts with and destroys NO (Figs. 3 & 4). This results in abnormally high NO consumption and the formation of reactive oxygen species, ultimately inhibiting vasodilation. NO destruction by hemoglobin can also cause further impairment in vascular endothelial function via transcriptional activation of adhesion molecules, including VCAM-1 and E-selectin, and potent vasoconstrictors such as endothelin-1.8 The simultaneous release of erythrocyte arginase during hemolysis12 will limit the availability of arginine to NOS, contributing to a deficiency of NO (Morris, 2008).

The relationship between increasing plasma hemoglobin levels and direct measures of decreasing NO-dependent blood flow or low NO bioavailability has been confirmed in many studies (Gladwin *et al*., 2010). All mouse models of hemolysis studied develop spontaneous PHT and right heart failure. Pathologic evaluations in these models find no chronic thrombosis in the pulmonary vasculature; rather, a functional impairment in NO signaling driven by NO scavenging by plasma hemoglobin. NO depletion, PHT, and systemic hypertension have been reported in animal studies by inducing intravascular hemolysis or by infusions of hemoglobin or hemolysate (Gladwin *et al*., 2010).

Free plasma hemoglobin, in addition to generating reactive oxygen species, such as the hydroxyl and superoxide radicals (through the Fenton and peroxidase and auto-oxidation chemical reactions) is also a potent scavenger of nitric oxide (Fig.3) (Gladwin and Vichinsky 2008).

c) Indirect Effect of Hemolysis on NO through Affecting Arginine:

Hemolysis also releases erythrocyte arginase 1 into plasma (Fig 3). Arginase redirects the metabolism of L-arginine to L-ornithine (L arginine is originally formed from L-ornithine), reducing the required substrate for nitric oxide synthesis and compounding the reduction in the bioavailability of nitric oxide in sickle cell disease. Arginase 1 modulated the metabolic profile of arginine by reducing arginine levels and increasing the production of ornithine relative to that of citrulline (Gladwin and Vichinsky 2008). In addition, arginine and ornithine compete for uptake via the same transport system, decreases in the arginine ornithine ratio also represent decreases in arginine bioavailability (Morris, 2005). These abnormalities lead to severe pulmonary hypertension (Fig. 4) (Gladwin and Vichinsky 2008).

The formation of polyamines and L-proline, is another consequent for redirection the metabolism of L-arginine to L-ornithine. Polyamines and L-proline are essential for smooth muscle cell growth and collagen synthesis. Therefore, the induction of arginase may also promote aberrant vessel wall remodeling and neointima formation (Fig. 4). Proline is an amino acid that is also involved in lung fibrosis, airway remodeling, and asthma in addition to vascular smooth muscle proliferation, common features of pulmonary dysfunction in SCD and thalassemia. In SCD, pulmonary complications compromise oxygenation and contribute to a vicious cycle of erythrocyte sickling. By creating a shift towards ornithine metabolism, arginase triggers a process that contributes to the proliferative vasculopathy commonly found in hemolytic disorders (Morris, 2008).

Oxidative Stress and Vasculopathy

a) Causes of Oxidative Stress in Hemolytic Anemias

Oxidative stress plays a major role in the pathophysiology of thalassemia, sickle cell and other congenital and acquired hemolytic anemias. Free extracellular (labile plasma iron, LPI) and intracellular (labile iron pool, LIP) iron species that have been identified in patients' blood cells are responsible for generation of oxidative stress by catalyzing formation of oxygen radicals over the antioxidant capacity of the cell (Fig.3) (Fibach and Rachmilewitz 2010).

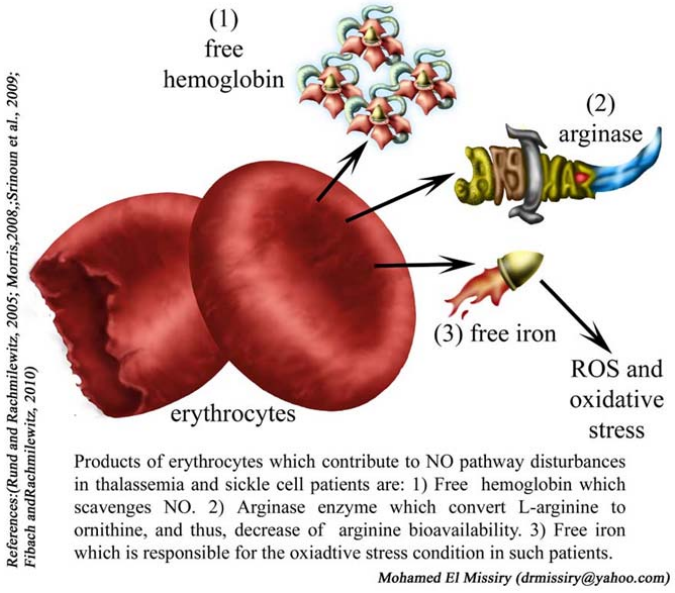

Fig. 3: Erythrocyte components and NO disturbances

b) Oxidative stress and Decrease of NO by Mean of Peroxynitrite Formation is Another Scavenging System for NO.

Oxidative stress is another prominent mechanism of vasculopathy. In hemolytic disorders, the erythrocyte may be a major determinant of the global redox environment. The sickle and thalassemia erythrocytes have increased concentrations of reactive oxygen species (ROS) compared with normal red blood cells. Overproduction of ROS, such as superoxide, by both enzymatic (xanthine oxidase, NADPH oxidase, uncoupled eNOS) and nonenzymatic pathways (Fenton chemistry), promotes intravascular oxidant stress, which is another potent scavenger of nitric oxide. besides the hemoglobin scavenging system (Gladwin and Vichinsky 2008).

This NO disruption and scavenging is happened by consuming of superoxides to the NO in the formation of the harmful potent oxidant peroxynitrite (Fig. 4). This superoxide consumption to NO is also augmented by increase xanthine oxidase expression. This, in turn, could lead to increased arginine metabolism in a compensatory attempt to produce more NO, thereby depleting arginine further during periods of heightened oxidative stress or hemolysis. Also, NO synthase may produce superoxide in lieu of NO when arginine concentration is low. Under such conditions, up-regulation of NO synthase could actually enhance oxidative stress. Indeed, studies in transgenic sickle-cell mice demonstrate that NO synthase activity is paradoxically increased (Morris, 2003, 2006 and 2008).

c)Uncoupled NOS and Shifting of Its Action from NO Synthesis to the Production of Superoxide

Hemolysis will drive arginine consumption, which will ultimately exacerbate NO sequestration and decreased NO synthesis. Under conditions of hypoxia, high asymmetric dimethyl arginine (ADMA), low arginine, or low availability of essential NOS cofactors (NADPH and/or tetrahydrobiopterin), NOS will be uncoupled, producing reactive oxygen species in lieu of NO, further reducing NO bioavailability and adding the milieu of oxidative stress. Consequently, NO synthase may produce superoxide in lieu of NO when arginine concentration is low leading to further decrease NO synthesis and exacerbating of NO deficiency. Under such conditions, up-regulation of NO synthase could actually enhance oxidative stress. Indeed, studies in transgenic sickle-cell mice demonstrate that NO synthase activity is paradoxically increased. (Fig. 4) (Morris, 2006 and 2008).

d) Glutation Pathway alteration Cause Further Restriction in Arginine Formation

Alterations in the glutathione buffering system are common to these hemoglobinopathies as an additional consequent of oxidative stress. These alterations in the glutathione buffering system may render erythrocytes incapable of handling the increased oxidant burden, thereby predisposing them to hemolysis. Recently it was discovered that a depletion of erythrocyte glutamine concentration and aberrations in erythrocyte glutathione metabolism is linked to severity of PHT in SCD and bio-markers of hemolytic rate. Glutamine, an essential precursor in NADPH biosynthesis, is metabolized to the glutathione substrate glutamate in the process of NADPH production. Glutamine thus plays an antioxidant role through preservation of intracellular NADPH, making it an important amino acid for glutathione homeostasis. Glutamine also serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway (Fig. 4) (Morris, 2008).

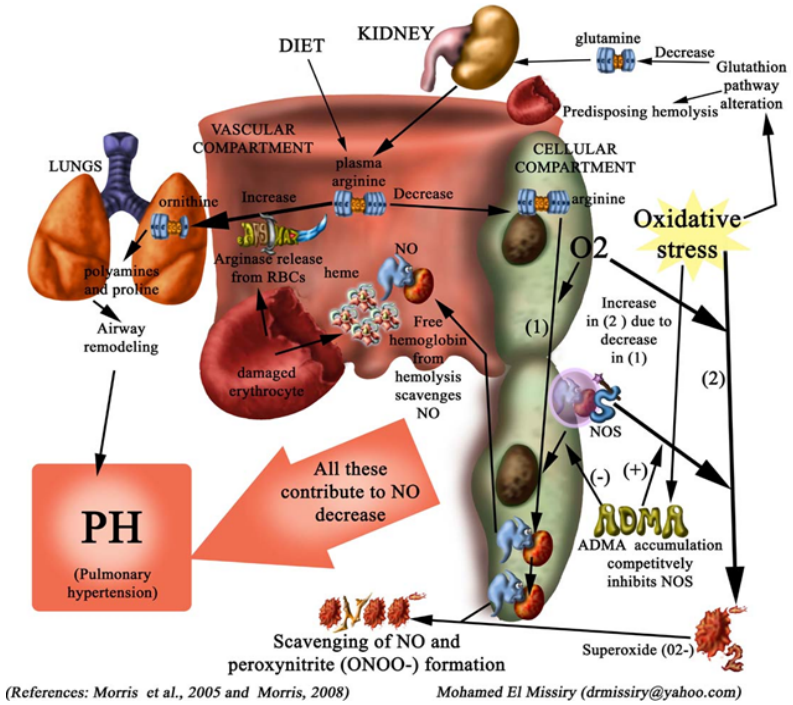

Fig. 4: Disturbed Arginine-NO Metabolism in Hemolytic Anemias.

e) ADMA: An Arginine Analog and Competitve Inhibitor of NOS

The normal function of the vascular system depends on nitric oxide (NO) production by vascular endothelial cells. However, under conditions associated with oxidative vascular injury, such as sickle cell disease excess formation of reactive oxygen species (ROS) can lead to an accumulation of asymmetric dimethyl arginine (ADMA). This accumulation of ADMA competitively inhibits all NO synthase isozymes as well as arginine transport, which in turn, decreases NO production. With limited L-arginine, NOS forms superoxide (O-), which causes vascular endothelial injury and further inflammation. The deleterious effects of low L-arginine availability may lead to a pro-atherosclerotic environment and subsequent cardiovascular disease (Fig. 4) (King *et al*., 2008, Morris, 2006).

Elevated plasma concentrations of asymmetric dimethylarginine (ADMA) contribute to limiting NO bioavailability in SCD. ADMA and symmetric dimethylarginine (SDMA) derive from the irreversible post-translational methylation of arginine residues by protein arginine methyltransferases (PRMT) and are released as free amino acids upon proteolysis. ADMA (but not SDMA) competitively inhibits NO synthase (NOS) enzymes, thereby limiting NO production. ADMA is degraded by dimethylarginine dimethylaminohydrolases (DDAH) whereas SDMA is mainly cleared renally. Circulating ADMA levels are elevated in several conditions of endothelial dysfunction, including SCD, and have been implicated in the pathophysiology of systemic and PH and risk of early mortality)Morris, 2006 and 2008; Landburg *et al*., 2008).

Coagulopathy and the Hypercoagulable State

Chronic depletion of nitric oxide and arginine may also contribute to the hypercoagulable state in hemolytic diseases. Since nitric oxide is a potent inhibitor of platelet activation, the depletion of nitric oxide and arginine (the substrate for nitric oxide synthesis) allows for platelet activation. Arginine consumption is compounded by increased intracellular platelet expression of arginase (Morris 2008; Gladwin and Vichinsky 2008).

Splenectomy is an additional risk factor for the development of PH and a hypercoagulable state in hemolytic disorders. Singer and colleagues recently reported an association of PH with splenectomy, increased platelet activation, hypercoagulability and evidence of chronic hemolysis in patients with both beta-thalassemia major and intermedia. High thrombin generation is known to occur in both SCD and thalassemia syndromes, as thrombin itself increases arginase activity in human endothelial cells (Morris 2008).

Suggested Agents for Increasing Nitric Oxide in Thalassemia and Sickle cell Anemia

L- Arginine:

Arginine is a nutritional supplement with very low toxicity and few adverse effects; it is well tolerated even at high doses (30–60g/day). It is one of the least toxic of the amino acids, and its efficacy has been tested in hundreds of human and animal trials for a variety of clinical indications. L-arginine intake varies according to important demographic and cardiovascular risk factors. Interventional studies with L-arginine have used widely divergent dose levels, however, average arginine intake from the American diet is typically 4–5 g/day (Evans *et al*., 2004; King *et al*., 2008).

In ten patients with SCD and PHT, oral arginine hydrochloride supplementation (100 mg/kg three times a day) produced a 15.2% mean reduction in pulmonary artery pressures estimated by Doppler echocardiography and improved venous oxygen saturation measured by co-oximetry after 5 days of treatment (figure 2). A significant improvement in shortening fraction was also noted (mean improvement >5%; $p = 0.008$), suggesting that arginine affected both the pulmonary and systemic vascular resistance (Morris, 2003 and 2006).

Oral arginine significantly increases serum arginine levels and NO metabolite levels (in both serum and exhaled air) in healthy adults. Supplementation with L-arginine may reverse the endothelial dysfunction associated with hypercholesterolemia, smoking (smokers), hypertension sickle cell and thalassemia. Both intravenous and oral arginine have been safely used in patients with pulmonary, renal and cardiovascular disease, as well as SCD (Tapiero *et al*., 2002; Morris, 2003 and 2006).

In Hydroxyurea (HU) treatment, which is a drug widely used in treatment of Sickle cell anemia and It has been additionally used in thalassemia management, (Yavarian *et al*.,2004; Dixit *et al*., 2005; Hoffbrand *et al*., 2006) NO generation is increased. However, This NO increase with HU therapy may reduce arginine levels even further. If altered arginine bioavailability is a mechanism contributing to endothelial dysfunction, interventions that increase NO production at the expense of increased arginine turnover without replenishing

the substrate for NO production may ultimately put patients at risk for the In development of PHT (Morris 2006).Many factors contribute to relative arginine bioavailability, including renal function, intracellular arginine transport function, localized pools of intracellular arginine, arginase activity, and the presence of NO synthase inhibitors such as methylated arginine derivatives These factors and disease severity associated with irreversible structural changes in the pulmonary vasculature may influence the effectiveness of arginine supplementation and account for the discrepant reports (Morris 2006).

B) L- Citrulline

Arginine precursor citrullin can also be beneficial in such hemolytic disorders. Citrulline is an alpha amino acid that is metabolized to L-arginine in the vascular endothelium, kidney and other cells. Oral citrulline has been found to be more effective than L-arginine in producing an increase in the blood levels of L-arginine; a dose of 3.8 g/m2 body surface area increases the peak concentration of L-arginine by 227% four hours after administration, compared to a 90% increase with the same dose of L-arginine. The explanation is that citrulline does not undergo intestinal or hepatic metabolism because it is not a substrate for arginase, and therefore does not induce its expression and activation. Consequently, L-citrulline holds promise in the treatment of endothelial dysfunction, and perhaps in cardiovascular disease, in which L-arginine deficiency and bioavailability of NO is involved (Orea-Tejeda *et al*., 2010).

C) Nebivolol

Additionally, and regarding to the state of oxidative stress with its consequent NO resistance and its conversion to harmful peroxynitrite (ONOO-) in these patients, in addition to the significant cardiac problems in these patients, further researches about Nebivolol administration is also required. The cardioprotective nebivolol is a third generation beta-blocker. It is highly selective for the beta1-adrenoceptor, and has additional nitric oxide-mediated vasodilating and antioxidant properties, along with a favourable metabolic profile (de Boer, 2007). Nebivolol decreased superoxide (O2–) and peroxynitrite (ONOO–) concentrations and restores NO bioavailability (Mason, 2005; de Nigris *et al*., 2008).

D) Antioxidants:

It is suggested to use antioxidant agents besides the previously discussed agents in the management of NO-pathway disturbance induced portal hypertension in these category of patients with hemoglobinopathies. Oxidative stress and reactive oxgygen species (ROS) plays a pivotal role in the pathological mechanism of thalassemia and sickle cell generally, and the mechanism of NO disturbance specifically. In these hemoglobinopathies, Free iron is the cause of this oxidative stress by catalyzing formation of oxygen radicals (Fenton reaction). In consequent, antioxidants could play a cardinal role in thalassmia and sickle cell management. Antioxidants in this case to could be subdivided to iron chelators to eliminate the free-iron species, which in this respect, act like antioxidants. In addition, antioxidants such as vitamin E, N-acetylcysteine and polyphenols are also capable of ameliorating increased oxidative stress parameters. Antioxidants may be more effective if used in combination and, their administration together with iron chelators, may provide a substantial improvement in the pathophysiology of hemolytic anemias (Rund and Rachmilewitz, 2005; Eleftheriou,2007; Fibach and Rachmilewitz, 2010).

Suggested novel or uncommonly used antioxidant iron chelators in these hemoglobin disorders could be:

I) Green tea:

Green tea shows many pharmacological effects, particularly antioxidative and iron-chelating capacities. A study by Srichairatanakool *et al*., 2006 showed that Green tea decreased plasma NTBI concentration and counteracted the increase of oxidative stress in both Fe(2+)-EDTA-treated human plasma and erythrocytes. Green tea is a bifunctional natural product that could be relevant for management of iron overload and oxidative stress.

II) Transferrin injections:

Transferrin is an extracellular antitioxidant in which its mechanism of work is achieved by iron binding (Weatherall and Clegg, 2001). A study by Li *et al*., 2010 said that transferrin injections normalized labile plasma iron concentrations, increased hepcidin expression, normalized red blood cell survival and increased

hemoglobin production; this treatment concomitantly decreased reticulocytosis, erythropoietin abundance and splenomegaly. These results indicate that transferrin is a limiting factor contributing to anemia in mice and suggest that transferrin therapy might be beneficial in beta-thalassemia,

III) Inositol 6 phosphate:

A study by Hawkins *et al*., 1993 concluded that the inositol 6 phosphate uniquely provides a specific interaction with iron to inhibit totally its ability to catalyse hydroxyl radical formation; and they suggested that a physiological function of inositol 6 phosphate might be to act as a safe binding site for iron during its transport through the cytosol or cellular organelles.

Suggested Natural Antioxidant with Additional Benefits (A Double Bladed Effect)

Resveratrol: Several observations lead to the conclusion that production of fetal hemoglobin (HbF) can functionally compensate for the defect of β-globin chain production in thalassemia and sickle cell (Bianchi *et al*.,2009) Consequently, Hydroxyurea is used as the classical HbF inducer in the treatment regimens of sickle cell and thatalssemia (Yavarian *et al*.,2004; Hoffbrand *et al*., 2006). It was discovered by Bianchi *et al*., 2009 that Resveratrol is a HbF inducer mimicking the biological activity of hydroxyurea in the induction of HbF production also it has a strong antioxidant activity (Bianchi *et al*., 2009).

Conclusion and Recommendations

Pulmonary hypertension (PHT) appears to be a critical problem of great impact in the pathological sequel of patients with thalasssemia and sickle cell anemia hemoglobinopathes. It was found that NO pathway disturbances play the major role PH of these diseases. Accordingly, the search for appropriate treatment aiming at correction of these disturbances is extremely essential.

L- arginine, L-citrullin and administration is suggested to be studied and used in researches for the treatment aiming at the correction of the state of NO disturbance in these patients. Performing researches and clinical trials are needed to evaluate them, recognize its beneficial impacts, and then we can determine their cost-benefit usage outcomes, and reach the most proper and effective doses and formulations to be applied for the benefit of this category of hemoglobinopathic patients at the end.

Studies concerning Nebivolol administration in these patients are additionally required, for its benefits in increasing NO synthesis with avoidance of the undesirable peroxynitrite, In addition to its cardioprotective effects in these patients with cardiovascular co-morbidities.

In the light of the cardinal role played by oxidative stress in NO-pathway disturbances; antioxidants is additionally suggested as a complementatary therapy in parallel with any of the previous discussed agents (L arginine, L-citrulline and Nebivolol) in treatment of thalassmemia and sickle cell patients, aiming at decreasing the oxidative stress burden in such patients.

References

Aessopos, A., M. Kati, D. Farmakis, 2007. Heart disease in thalassemia intermedia: a review of the underlying pathophysiology. Haematologica, 92: 658-65.

Allen, B.W., C.A. Piantadosi, 2006. How do red blood cells cause hypoxic vasodilation? The SNO-hemoglobin paradigm. Am J Physiol Heart Circ Physiol., 291(4): H1507-12. Epub 2006 Jun 2.

Ataga, K.I., C.G. Moore, C.A. Hillery, S. Jones, H.C. Whinna, D. Strayhorn, C. Sohier, A. Hinderliter, L.V. Parise, E.P. Orringer, 2008. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. Haematologica, 93(1): 20-6.

Bianchi, N., C. Zuccato, I. Lampronti, M. Borgatti and R. Gambari, 2009. Fetal Hemoglobin Inducers from the Natural World: A Novel Approach for Identification of Drugs for the Treatment of β-Thalassemia and Sickle-Cell Anemia. Evid Based Complement Alternat Med., 6(2): 141-151.

Camaschella, C. and M.D. Cappellini, 1995. Thalassemia intermedia. Haematologica, 80: 58-68.

Cappellini, M.D., A. Cohen, A. Piga, J. Porter and A. Taher, 2008. Guidelines For The Clinical Management of Thalassaemia. 2nd revised edition (Thalassaemia International Fedration, Nicosia, Cyprus).

Dana, E., M.D. King,, G. Arch, I.I.I. Mainous, PhD., and E. Mark, 2008. Geesey, Ms.variation in L-arginine Intake According to Demographic Characteristics and Cardiovascular Risk. Nutr Res., 28(1): 21-24.

de Boer, R.A., A.A. Voors, D.J. van Veldhuisen, 2007. Nebivolol: third-generation beta-blockade. Expert Opin Pharmacother, 8(10): 1539-50.

de Nigris, F., M. Rienzo, C. Schiano, C. Fiorito, A. Casamassimi, C. Napoli, 2008. Prominent cardioprotective effects of third generation beta blocker nebivolol against anthracycline-induced cardiotoxicity using the model of isolated perfused rat heart. Eur J Cancer, 44(3): 334-40. Epub 2008 Jan 14.

Dixit, A., T.C. Chatterjee, P. Mishra, D.R. Choudhry, M. Mahapatra, S. Tyagi, *et al*., 2005. Hydroxyurea in thalassemia intermedia-a promising therapy. Ann Hematol., 84: 441-6.

Eleftheriou, A., 2007. About Thalassemia. Thalassemia International Publications (4).

Erdem, E., G. Bozdogan, G. Yanikkkaya Demirel and Y. Yildirmak, 2009. Periphral blood lymphocyte subsets, T-reg cells and FOXP3 expression in β- thalassemia major and β- thalassemia trait patients. Haematologica, 94[suppl.2]: 10 abs. 0025.

Evans, R.W., J.D. Fernstrom, J. Thompson, *et al*., 2004. Biochemical responses of healthy subjects during dietary supplementation with L-arginine. J Nutr Biochem., 15: 534-9.

Fibach, E., E.A. Rachmilewitz, 2010. The role of antioxidants and iron chelators in the treatment of oxidative stress in thalassemia. Ann N Y Acad Sci., 1202: 10-6.

Gladwin, M.T., R.J. Barst, O.L. Castro, V.R. Gordeuk, C.A. Hillery, G.J. Kato, D.B. Kim-Shapiro, R. Machado, C.R. Morris, M.H. Steinberg, E.P. Vichinsky, 2010. Pulmonary hypertension and NO in sickle cell. Blood, 116(5): 852-4.

Gladwin, M.T., 2008. Vichinsky E.Pulmonary complications of sickle cell disease. N Engl J Med., 359(21): 2254-65.

Hawkins, P.T., D.R. Poyner, T.R. Jackson, A.J. Letcher, D.A. Lander, R.F. Irvine, 1993. Inhibition of iron-catalysed hydroxyl radical formation by inositol polyphosphates: a possible physiological function for myo-inositol hexakisphosphate. Biochem J., 294(Pt 3): 929-34.

Hoffbrand, A.V., P.A.H. Moss and I.E. Pettit, 2006. Essential Haematology, 5th edition, Published by Blackwell Publishing Ltd.

King, D.E., A.G. Mainous, M.E. Geesey, 2008. Variation in L-arginine intake follow demographics and lifestyle factors that may impact cardiovascular disease risk. Nutr Res., 28(1): 21-4.

Knowles, R.G., S. Moncada, 1994. Nitric oxide synthases in mammals. Biochem J., 298(Pt 2): 249-58.

Landburg, P.P., T. Teerlink, E.J. van Beers, F.A. Muskiet, M.C. Kappers-Klunne, J.W. van Esser, M.R. Mac Gillavry, B.J. Biemond, D.P. Brandjes, A.J. Duits, J.J. Schnog, 2008. CURAMA study group. Association of asymmetric dimethylarginine with sickle cell disease-related pulmonary hypertension.Haematologica. 93(9): 1410-2. Epub 2008 Jul 18.

Li, H., A.C. Rybicki, S.M. Suzuka, L. von Bonsdorff, W. Breuer, C.B. Hall, Z.I. Cabantchik, E.E. Bouhassira, M.E. Fabry, Y.Z. Ginzburg, 2010. Transferrin therapy ameliorates disease in beta-thalassemic mice. Nat Med., 16(2): 177-82.

Lichtman, M., E. Beutler, K. Kaushansky, T. Kipps, U. Seligsohn, J. Prchal, 2007. Williams Hematology, Seventh Edition.

Mason, R.P., L. Kalinowski, R.F. Jacob, A.M. Jacoby, T. Malinski, 2005. Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. Circulation, 112(24): 3795-801. Epub 2005 Dec 5.

Mateo, R.D., G. Wu, F.W. Bazer, J.C. Park, I. Shinzato, S.W. Kim, 2007. Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr., 137(3): 652-6.

Morris, C.R., Kato G.J., Poljakovic M., Wang X., Blackwelder W.C., Sachdev V., Hazen S.L., Vichinsky E.P., Morris S.M. Jr, Gladwin M.T., 2005. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA., 294(1): 81-90.

Morris, C.R., S.M. Morris Jr, W. Hagar, *et al*., 2003. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? Am J Respir Crit Care Med., 168: 63-9.

Morris, C.R., E.P. Vichinsky, 2010. Pulmonary hypertension in thalassemia. Ann N.Y. Acad Sci., 1202: 205-13.

Morris, C.R., 2008. Mechanisms of vasculopathy in sickle cell disease and thalassemia. Hematology Am Soc Hematol Educ Program, 177-85.

Morris, C.R., 2006. New strategies for the treatment of pulmonary hypertension in sickle cell disease : the rationale for arginine therapy. Treat Respir Med., 5(1): 31-45.

Morris, C.R., 2010. Role of Arginase in Sickle Cell Lung Disease and Hemolytic Anemias, The Open Nitric Oxide Journal, 2: 41-54.

Olivieri, N.F., 1999. The ß-Thalassemias. N Engl J Med., 341(18): 1407.

Orea-Tejeda, A., J.J. Orozco-Gutiérrez, L. Castillo-Martínez, C. Keirns-Davies, P. Montano-Hernández, O. Vázquez-Díaz, A. Valdespino-Trejo, O. Infante, R. Martínez-Memije, 2010. The effect of L-arginine and citrulline on endothelial function in patients in heart failure with preserved ejection fraction. Cardiol J.,

17(5): 464-70.
Rund, D., E. Rachmilewitz, 2005. Beta-thalassemia. N Engl J Med., 353(11): 1135-46.
Srichairatanakool, S., S. Ounjaijean, C. Thephinlap, U. Khansuwan, C. Phisalpong, S. Fucharoen, 2006. Iron-chelating and free-radical scavenging activities of microwave-processed green tea in iron overload. Hemoglobin, 30(2): 311-27.
Srinoun, K., S. Svasti, W. Chumworathayee, J. Vadolas, P. Vattanaviboon, S. Fucharoen, P. Winichagoon, 2009. Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. Haematologica, 94(9): 1211-9.
Tapiero, H., G. Mathé, P. Couvreur, K.D.I. Tew, 2002. Arginine. Biomed Pharmacother, 56(9): 439-45.
Tripatara, A., A. Jetsrisuparb, J. Teeratakulpisarn and K. Kuaha, 2007. Hemostatic alterations in splenectomized and non splenectomized patients with beta-thalassemia/haemoglobin E disease. Thromb Res, Prepublished 2007 as dol:10.1016/j.thromres
Weatherall, D.J. and J.B. Clegg, 2001. The Thalassaemia Syndromes, Fourth edition.
Yavarian, M., M. Karimi, E. Bakker, C.L. Harteveld and P.C. Giordano, 2004. Response to hydroxyurea treatment in Iranian transfusion-dependent beta-thalassemia patients. Haematologica, 89: 1172-8.