Sickle cell disease and folate supplementation

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Abstract
Sickle Cell Disease is an important inherited blood disorder in which anemia occurs due to short life span of the deformed RBCs. Though SCD is predominantly present in Africa, it has been reported from other tropical regions including India. SCD may also manifest as vaso-occlusive crisis which occurs as a result of interplay among impaired blood rheology, increased adhesiveness of RBCs with inflammatory cells and vascular endothelium, and hemostatic activation. Damage of erythrocyte membranes also increases exposure of adhesion molecules and binding motifs viz. phosphatidyl serine, basal cell adhesion molecule-1/Lutheran, integrin-associated protein, and intercellular-adhesion-molecule-4. Release of immature RBCs or reticulocytes with adhesion molecules and increased cellular effect of selectins P- and E-, vascular-cell-adhesion-molecule-1, ICAM-1 and interleukin-8 on endothelial cells aggravates the crisis. Increased level of circulating homocysteine causes increased cytotoxic activity on endothelial cells, elevates hydrogen peroxide levels, decreases nitric oxide synthesis, induces cytokine production to stimulate the inflammatory state, activates procoagulant factors, and dysregulation of lipid metabolism. Positive role of folic acid supplementation in SCD is not well supported and there are possible side effects of folate supplementation. The final biologically active form of folic acid, L-Methylfolate or Levomefolic acid or 5-MTHF, is the best option which gets readily absorbed and exerts its action without requiring any bioconversion.

Keywords: Sickle cell disease, Vaso-occlusive crisis, 5-MTHFR, Folate supplementation.

Introduction
Sickle cell disease (SCD) is an important inherited blood disorder. In a patient with SCD, the haemoglobin S in the RBCs gets altered leading to a rigid sickle or half-moon shaped disfiguration of the RBCs which lose their plasticity and can choke the narrow blood vessels thereby hindering oxygen supply to different tissues/organs. The short life span of these RBCs gives rise to anaemia commonly known as Sickle Cell Anaemia. Lack of blood/oxygen supply to different tissues/organs in SCD leads to chronic severe pain (back, chest, hands and feet), bacterial infections, damage to bone/muscles/organs, and even necrosis. SCD may also lead to Sickle Cell Crisis (or Sickling Crisis) namely vaso-occlusive crisis, sequestration crisis, aplastic crisis and haemolytic crisis. Though the signs of SCD may appear in the childhood, its severity varies from one individual to another. Factors such as stress, excessive exercise, dehydration, temperature variation (cold climate) and high altitude often play important role in setting in a crisis.[1]

Epidemiology
Though SCD is predominantly present in Africa, it has been recorded in the population of other tropical regions viz. Arabian Peninsula, and central, southern and eastern parts of India. Population migration from Africa has also led to reporting of this condition from other countries as well. Sub-Saharan Africa is believed to have about 80% of SCD reported globally.[2] As per a Global Burden of Disease (GBD) report of 2015, SCD affected about 4.4 million people and an additional population of 43 million had Sickle Cell Trait.[3,4] In 2015, it resulted in about 1,14,800 deaths.[5] A significant prevalence of the mutation responsible for sickle cell has been reported among other ethnic groups such as those native to Italy, Greece, Turkey, Saudi Arabia, India, Pakistan, Bangladesh, China, and Cyprus.[6] WHO has reported that the prevalence rate of SCD varies between 20-30% in Cameroon, Ghana, Nigeria, Republic of Congo and Gabon, and about 45% in some parts of Uganda.[7] According to the above WHO report, about 5% of the world population carries the trait genes of haemoglobin disorders (SCD and thalassemia) and about 3,00,000 babies with severe haemoglobin disorder are born each year.

In India, Lehman and Cutbush[8] first reported sickle haemoglobin in tribal population in the Nilgiris in south India in the year 1952. Dunlop and Mazumder[9] in the same year reported similar findings in the tea garden migrant laborers from Bihar and Odisha in Assam. A large number of subsequent screening studies have shown that the ethnic/tribal population mostly present in the States of Madhya Pradesh, Maharashtra, Odisha, Gujarat, Chhattisgarh and certain pockets of Tamil Nadu and Kerala are sickle cell carriers.[10] SCD is commonly encountered in the ethnic population of central India who share a genetic linkage with African communities.[11] In endemic areas of Madhya Pradesh, Rajasthan and Chhattisgarh, the prevalence varies between 9.4 to 22.2%.[12] Screening of new born babies for the presence of sickle cell disorders in the population has been initiated in the states of Gujarat, Maharashtra, Madhya Pradesh, Chhattisgarh and Odisha.[13-16]

Vaso-occlusive phenomenon and role of homocysteine
Polymerization of mutant haemoglobin S and impairment of RBC rheology occur due to a single amino acid substitution in the beta-globin chain. Abnormalities in RBCs result in haemolysis and a vicious cycle of vaso-occlusive phenomenon which in turn triggers inflammation and redox instability finally leading to progressive small- and large-vessel vasculopathy.[17] Recent studies have shown that vaso-

occlusion occurs as a result of interplay among impaired blood rheology, increased adhesiveness of RBCs with inflammatory cells and vascular endothelium, and haemostatic activation.[18] Blood rheology is guided by plasma viscosity, haematocrit and RBC deformability. Increased plasma viscosity is an important factor in reduced blood flow through capillaries and venules of tissues.[19] These deformable sickle cells may further get mechanically sequestered in the microcirculation leading to transient vaso-occlusion.[2,20] Damage of erythrocyte membranes also increases exposure of adhesion molecules and binding motifs viz. phosphatidyl serine (PS), basal cell adhesion molecule-1/Lutheran (B-CAM-1/L), integrin-associated protein (IAP), and intercellular-adhesion-molecule-4 (ICAM-4).[19-21] Further, as a result of chronic anaemia in SCD, immature RBCs or reticulocytes with adhesion molecules (VLA-4 and CD 36) are released in the circulation.[21] Endothelial dysfunction and sterile inflammation also increase the cellular effect of selectins (P- and E-), vascular-cell-adhesion-molecule-1 (VCAM-1), ICAM-1 and chemoattractant like interleukin-8 (IL-8) on endothelial cells.[18,22,23] Various studies have shown the role of erythrocyte-neutrophil-endothelium or platelet-neutrophil-endothelium adhesions in microcirculation as a cause of systemic vaso-occlusion. Further, the cellular and molecular mechanisms of vaso-occlusion are also dictated by the type of organ or vascular bed.[17]

Enzyme methionine synthase uses 5-Methyletetrahydrofolate (MTHF) to convert homocysteine (a sulfur containing toxic amino acid) to methionine, and hence, a deficiency of 5-MTHF will lead to increased level of circulating homocysteine. Homocysteine when present in high concentration in plasma, it acts as a risk factor for cardiovascular disease, stroke, venous thrombosis and arteriosclerosis.[24,25] High concentration of homocysteine causes increased cytotoxic activity on endothelial cells, elevates hydrogen peroxide levels, decreases nitric oxide synthesis, induces cytokine production to stimulate the inflammatory state, activates procoagulant factors, and dysregulation of lipid metabolism. Hyperhomocysteinemia is also responsible for changes in rheological properties of blood viz. decreasing antithrombin III and tissue plasminogen activator, and increasing factor VII and C-protein.[26,27] Further, homocysteine also increases interaction between endothelial cells and leukocytes.[28] Studies have found higher plasma homocysteine concentration in SCD patients in spite of higher plasma folate and vitamin B12 concentration.[29]

Synthesized in endothelial cells, nitric oxide (NO), regulates vasal vascular tone and endothelial function, and maintains blood oxygenation via hypoxic pulmonary vasoconstriction and reduced shunt physiology. NO also has vaso-dilatory, antioxidative, anti-adhesion and anti-thrombotic properties. Hence, any imbalance in the NO homeostasis could adversely affect the SCD pathophysiology.[30]

Cytokine expression increases in vaso-occlusive and proinflammatory episodes. This phenomenon reflects a positive correlation to the increase in dehydration in SCD.[31,32] These cytokines stimulate a membrane oxidoreductase (protein disulfide isomerase), which exists in higher concentrations in sickle-RBC membranes compared with those on healthy RBCs.

SCD patients exhibit high levels of thrombin generation markers, reduction of natural anticoagulant proteins, thrombotic complications, and increase in platelet activation, fibrinolytic system and tissue factor expression. Thus, coagulation activation is multi-factorial with contributions from ischemia-perfusion injury and inflammation, hemolysis and NO deficiency, and increased RBC phosphatidylserine expression.[33]

Folic acid supplementation

Because of premature destruction of the RBCs in SCD patients, RBC count is always lower than normal and folate stores are often depleted because of high cell turnover. This requirement of folic acid for erythropoiesis is compensated by the dietary intake. Folate acts as a coenzyme in the synthesis of nucleic acids including the conversion of homocysteine to methionine and the methylation of deoxyuridylate to thymidylate. During DNA synthesis, folate is required for proper cell division, the impairment of which can lead to megaloblastic anaemia.[34-36]

MTHF is a member of the group of compounds known as ‘folate’ and is the primary form found in serum. Folate plays an important role in regulating homocysteine concentration and hence, it is indicated in cases of hyper-homocysteinemia. In the digestive system, the majority of dietary folate is converted into 5-MTHF before entering the bloodstream. Though Folic acid supplementation treats chronic haemolytic anaemia, its positive role in SCD is not well supported. Folate intake leads to a decrease in symptoms of anaemia in SCD and Folic acid replenishes the depleted folate stores necessary for erythropoiesis. Potential advantages of folate therapy in patients with SCD include the prevention of hyper-homocysteinemia but that may predispose to thrombotic events.[37] It is believed that folate in anaemia raises haemoglobin levels and helps provide a healthy reticulocyte response.[38] In patients with SCD, folate supplementation does not improve the deficiency or megaloblastic changes and folic acid supplementation did not improve the serum and erythrocyte folate levels.[39] One study found no “striking effects” of folic acid supplementation in sickle cell anaemia on the hematological profile or on growth in children with SCD who received this nutrient.[40]

Folic acid supplementation @ 1mg/day for patients with Sickle cell anaemia has been recommended in the Guidelines for SCD by the National Heart, Lung, and Blood Institute.[41] Literature reviews by Yasin et. al. (2012)[39] revealed that there are studies favouring folic acid supplementation. These studies have cited low serum and erythrocyte folate levels in SCD patients and high incidence of megaloblastic anaemia, and the positive effects of supplementation included reversal of developmental delay, reduced dactylitis and reduction of homocysteine levels

leading to reduced cardiovascular, stroke and venous thrombosis risk. Yasin et. al[39] also came across studies against folic acid supplementation which conversely found that folate is not deficient in patients, megaloblastic change is uncommon and both these parameters did not improve with supplementation. Also, folic acid does not increase haemoglobin, growth characteristics, infections, splenic sequestration and dactylitis.

Possible side effects of folate supplementation include increased priapism and increased twin pregnancy rates in patients with SCD,[39] an increased risk of some neoplasms like colorectal carcinoma with high folate intake[42] and a detrimental effect on cancer-protective natural killer cells.[43] Some research has found that folate supplementation in SCD can mask cobalamin deficiency with consequent neuropsychiatric manifestations.[44]

Unlike most folate, the majority of folic acid is not converted to the active form of vitamin B9, 5-MTHF, in the digestive system. Instead, it needs to be converted in the liver or other tissues.[45,46] Even a small dose, such as 200–400 mcg per day, may not be completely metabolized until the next dose is taken. This is a cause for concern, since high levels of un-metabolized folic acid have been associated with several health problems including increased cancer risk. These may also speed up growth of precancerous lesions.[47-49] In elderly people, high levels of folic acid may mask vitamin B12 deficiency and untreated vitamin B12 deficiency may lead to dementia and impair nerve function.[50,51] Some studies have found that circulating unmetabolized folic acid is linked to reduced natural killer cell activity – an important part of the innate immune system.[43]

Various signs of folic acid accumulation in circulating blood may include nausea, decreased appetite, bloating, disturbed sleep, feeling irritable, numbness and or tingling, oral sores, skin rashes, psychological behaviour and even seizures. Hence, the rationale of administering folic acid as a treatment in SCD (where the patient is already deficient in 5-MTHFR enzyme) is highly debatable. However, the final biologically active form of folic acid, L-Methylfolate or Levomefolic acid or 5-MTHF, is the best option which gets readily absorbed and exerts its action without requiring any bioconversion. This is most suitable even in the SCD patients who already have MTHFR deficiency. L-methylfolate is the primary biologically active form of folate used at the cellular level for DNA reproduction, the cysteine cycle and the regulation of homocysteine. It is also the form found in circulation and transported across membranes into tissues and across the blood-brain barrier. It is synthesized in the absorptive cells of the small intestine from polyglutamylated dietary folate. It is a methylated derivative of tetrahydrofolate. Levomefolic acid is generated by MTHFR from 5, 10- methylenetetrahydrofolate (MTHF) and used to recycle homocysteine back to methionine by methionine synthase.[52] L-methylfolate is water-soluble and primarily excreted via the kidneys. In a study of 21 subjects with coronary artery disease, peak plasma levels were reached in one to three hours following oral or parenteral administration. Peak concentrations were found to be more than seven times higher than folic acid.[53]

Conclusion

Vaso-occlusive crisis is an important consequence of SCD. Homocysteine when present in high concentration in plasma, it acts as a risk factor for cardiovascular disease, stroke, venous thrombosis and arteriosclerosis. Folate plays an important role in regulating homocysteine concentration and hence, it is indicated in cases of hyper-homocysteinemia. Potential advantages of folate therapy in patients with SCD include the prevention of hyper-homocysteinemia but that may predispose to thrombotic events. Folic acid supplementation @ 1mg/day for patients with Sickle cell anaemia has been recommended. However, owing to the possible side effects of folate supplementation, the final biologically active form of folic acid, L-Methylfolate or Levomefolic acid or 5-MTHF, is preferred. It gets readily absorbed and exerts its action without requiring any bioconversion. This is most suitable even in the SCD patients who already have MTHFR deficiency.

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Conflict of interest

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